VOLUME 41 • ISSUE 4 • OCTOBER - DECEMBER 2022

Achaiki Iatriki

OFFICIAL PUBLICATION OF THE MEDICAL SOCIETY OF WESTERN GREECE AND PELOPONNESUS

ISSN: 1106-3319
ISSN (ON LINE): 1792-3018

ACHAIKI IATRIKI

OFFICIAL JOURNAL OF THE MEDICAL SOCIETY OF WESTERN GREECE AND PELOPONNESUS (IEDEP)

GENERAL INFORMATION

ISSN Print Edition: 1106-3319
Journal Homepage: http://www.iedep.gr/
ISSN Electronic Edition: 1792-3018
NLM Unique ID: 9802550

Journal citation: *Achaiki Iatriki* is published on behalf of the Journal of the Medical Society of Western Greece and Peloponnesus (IEDEP), representing the Society's official Journal. Please cite articles of the Journal as: Author names. Title of article. Ach Iatriki year;volume:pages.

Aims and scope: The journal publishes original papers on clinical and basic research from all areas of the health sciences including healthcare. *Achaiki Iatriki* is an open access journal. It provides immediate free access to its scientific contents and authors are not charged for submission, processing or publication of the manuscripts.

Abstracting and indexing services: Achaiki Iatriki is abstracted/indexed in the following databases: Google Scholar and Index Copernicus.

MEDICAL SOCIETY OF WESTERN GREECE AND PELOPONNESUS

42 Votsi Street, Patras 26221, Greece
Tel: +30 2610 279579, Fax: +30 2610 220518
email: iede_pel@yahoo.gr

Publisher
Medical Society of the Western Greece and Peloponnesus

Editor-in-Chief
Christos Triantos
email: achaiki.iatriki@gmail.com

ACHAIKI IATRIKI

OFFICIAL JOURNAL OF THE MEDICAL SOCIETY OF WESTERN GREECE AND PELOPONNESUS (IEDEP)

ACHAIKI IATRIKI

OFFICIAL JOURNAL OF THE MEDICAL SOCIETY OF WESTERN GREECE AND PELOPONNESUS (IEDEP)

ACHAIKI IATRIKI

OFFICIAL JOURNAL OF THE MEDICAL SOCIETY OF WESTERN GREECE AND PELOPONNESUS (IEDEP)

ACHAIKI IATRIKI

Quarterly Official Journal of the
Medical Society of Western Greece And Peloponnesus (IEDEP)

CONTENTS

Dear colleagues,

In the current issue, the editorial by Lampri E., describes the role of the collaboration between a pathologist and a gastroenterologist and emphasizes the need for a direct and close communication for the benefit of the patient.

Moreover, this issue includes five reviews. The first review, by Bellou et al. summarizes the latest data regarding the acute phase and the long-term treatment of the pulmonary embolism. The review by Dimitrakopoulos et al. describes the signaling pathways involved in metastatic colorectal cancer development and progression and identifies the molecular targets that constitute eligible targets for immunotherapy drugs. The review by Konstantopoulou et al. provides an overview of biosensors development and their applications across several domains. The review, by Liossis et al. presents data on the direct and indirect role of B cells in three autoimmune disorders: systemic lupus erythematosus, rheumatoid arthritis and systemic sclerosis. Lastly, the review by Staveri C., describes novel therapeutic approaches regarding the Systemic Lupus Erythematosus, focusing particularly on lupus nephritis.

Warmest wishes for a happy holiday season,
Yours sincerely,

C. Triantos
Associate Professor in Internal Medicine
and Gastroenterology Faculty of Medicine,
School of Health Sciences, University of Patras
Editor-in-Chief of the journal "ACHAIKI IATRIKI"

ACHAIKI IATRIKI | 2022; 41(4):171–174

How important is the collaboration among gastroenterologists and pathologists? Do they see two sides of the same coin?

Evangeli Lampri

Improving communication among healthcare providers is a familiar topic to most physicians. Among them, gastroenterologists and pathologists have realized that their effective communication must become an even higher priority. Although this concept may seem to receive a disproportionate amount of emphasis, research continues to show that poor communication contributes to many medical errors and is "a top reason for team mishaps and subsequent lawsuits" [1,2]. This working relationship often requires close collaboration and coordination; thus, successful communication is vital to ensure patient safety and reduce the risk of errors.

A patient, who experiences uncomfortable symptoms involving his digestive tract, is going to contact a gastroenterologist. The forementioned clinician must take an accurate history, perform a physical examination and if it is necessary a diagnostic endoscopy, taking biopsies. Then, the ball goes to the pathologist's field. Even the most experienced pathologist may find it extremely difficult to report a case without being aware of the clinical history of the patients. The information that a pathologist must know in order to appropriately evaluate a biopsy and make a diagnosis is diverse, and usually the main source of this information is the gastroenterologist, such as every other clinician responsible for the care of patients.

The pathologist will study the cytological and histological structure of normal or abnormal tissue. The pathologist's report will have major implications for the clinical diagnosis, management, and follow up. Then, the gastroenterologist will play a continuing role in the treatment and well-being of the patient after the diagnosis [3,4].

Patients may never meet the pathologist involved in diagnosis, but an accurate and detailed diagnosis is a critical first step to move forward and define a treatment plan.

The gold standard for diagnosis is the bidirectional relationship between the pathologist and the gastroenterologist.

In general, good diagnosis is based on the following procedures: clinical examination, endoscopy, sampling (biopsy), morphological evaluation, and reporting [3,4].

The endoscopist must provide the pathologist with information about the patient, including the findings of the gross examination, biopsy location, relevant clinical history, bowel preparation, and current medications [5]. For example, a gastroenterologist should clarify whether a lesion is local or diffused, because that may help, for example, in the differential diagnosis of an "inflammatory polyp" from "colitis".

Moreover, the gastroenterologist is responsible for supplying appropriate samples. One study found that the number of biopsy samples from two to eight improved the detection of esophageal carcinoma from 95.8% to 100%, meaning that four cases out of 100 are missed if only two biopsy samples are taken [6].

Consider a pathologist with a gastric biopsy, not being aware of the patient's history of gastric MALT lymphoma. He may consider it as chronic gastritis,

Department of Pathology, Faculty of Medicine, Scool of Health Sciences, University of Ioannina, Ioannina, Greece
Received: 30 Sep 2021; Accepted: 14 Jun 2022

Key words: *Gastroenterologist; pathologist; biopsy; diagnosis*

missing a remaining focus, which sometimes needs immunohistochemistry to be revealed.

There are cases of Inflammatory Bowel Disease (IBD) where there are no isolated histological features diagnostic of a subtype of IBD. Instead, the pathologist should take into consideration that certain features are more prevalent in one subtype than in another. Diagnostic accuracy is optimized if there is the opportunity to assess multiple features together, for example if the intersite and intrasite distribution of changes is also analyzed, and if clinical details are taken into account [7]. A diagnosis of IBD is a challenging task for a pathologist as he/she cannot do it by his/her own. He must know the number of biopsy specimens taken, the topography of the samples, the endoscopic report, the macroscopic appearance of the mucosa, the endoscopic score of the inflammation [8,9]. The extent of the disease can be determined endoscopically. Moreover, in a long standing IBD, the pathologist must be aware of any treatment which may have changed the course of the disease [10-12].

A gastroenterologist can get the best out of his pathologist, giving the appropriate information about the patient's clinical history, making him/her aware of the possible clinical diagnosis and asking him/her to collaborate in order to conclude the most accurate diagnosis for the patient.

Pathologists have to talk to their clinicians and vice versa; sometimes a case may need to be discussed in a multidisciplinary team, for example a case of IBD with extensive areas of dysplasia. All these recommendations may seem obvious and undisputable, however most of pathology departments receive samples with incomplete or no clinical details, or even worse biopsies from different sites may arrive within the same vial [11, 12].

On the other hand, biopsies may be interpreted wrongly when pathologists are unaware of the clinical background. Pathologists should examine and describe only features that are relevant to the clinician and reproducible. Histopathological findings must be reported in an accurate, reliable and reproducible way. The language must facilitate clear, direct communication among pathologists themselves and between pathologists and gastroenterologists [3, 4]. The pathology report must be clear and comprehensive for clinicians, describing all histological features and providing a diagnosis. The best scenario would be the lowest interobserver and intra-observer differences.

The most common clinical decisions, based on pathological findings, involve the differential diagnosis between malignant and benign lesions, as well as the characterization of inflammation in IBD, gastritis etc. Based on histopathological diagnosis, a gastric or colonic polyp may be benign or neoplastic [13, 14]. A biopsy from the terminal ileum can differentiate Crohn's ileitis from tuberculosis [15] and a colonic biopsy distinguishes ulcerative colitis from specific, self-limited colitis, or Crohn's disease [16].

Both pathologists and gastroenterologists must cooperate and use common terminology and follow accepted guidelines, such as the Sidney classification of gastritis [17] and low grade versus high grade dysplasia in Barrett's esophagus [18] and ulcerative colitis [19]. The pathologist's diagnosis determines patient management, follow up, and treatment. For example, a diagnosis of Barrett's esophagus needs an annual or biennial endoscopy and biopsy, and treatment with high dose proton pump inhibitors. If the diagnosis is that of Barrett's esophagus with low grade dysplasia, the patient needs endoscopy after six months, but when the diagnosis is high grade dysplasia, endoscopic mucosal resection or surgery should be performed [18].

The pathologist must accurately communicate the results and provide all necessary data so that the gastroenterologist can take the necessary steps for treatment or follow up. There are cases with uncertainties in diagnosis [3, 4]. Pathologists often use terms as "consistent with" or "suggestive of" which can be interpreted differently by different people [20]. A scoring system would help avoid any misunderstanding or confusion, but this is not always possible [21]. For example, an adenoma of the large intestine should be characterized as having, low or high grade, dysplasia and not with descriptive terms with no clinical applications. The pathologist should try to reach a conclusion and not only a descriptive diagnosis, as this may be misleading. Describing mild chronic inflammation in colon mucosa which may be a feature of normal colon without clinical importance may lead to unnecessary follow up, as gastroenterologists may suspect the beginning of a colitis. Moreover, the inclusion of the term "atypia" in a pathology report should be clarified whether it refers to regenerative or dysplastic. The "grey zones" of unclassified dysplasia should be avoided, if it is possible.

The pathologist must provide a reproducible and useful report that addresses the clinical questions posed by the endoscopist. A poor interdisciplinary dialogue can lead to mistreatment or mismanagement, some-

times with dire outcome. Histopathological evaluation is prone to subjective biases, despite the use of indices. In addition, these indices are developed by expert IBD pathologist, but applied at large, by general pathologist [22].

Gastroenterologists and pathologists should consider themselves as the blind men in the parable with the elephant. Imagine these blind men who have never come across an elephant before and try to learn what the elephant is like by touching it. Each blind man examines a different part of the elephant's body, but only one part, such as the side or the tusk or the tail. Then they describe the elephant based on their limited experience. The moral of the parable is that doctors should not claim absolute truth based only on the experience of their specialty, ignoring other doctors' experiences.

A more sophisticated or detailed diagnosis actually translates to better care, and provides numerous examples that show not only a clinical benefit to the patient and the gastroenterologist, but also a financial advantage for payors (patients or insurances) [12]. For the optimal communication between pathologists and gastroenterologists, pathologists must ensure accurate assessment and clear and relevant reports, and gastroenterologists must provide all relevant clinical information, the endoscopic picture and ensure proper and adequate sampling. The coin is the same: the ultimate benefit of the patient.

Conflict of interest disclosure: *None to declare*

Declaration of funding sources: *None to declare*

Author contributions: *Evangeli Lampri: conception, writing, data interpretation and review of the final draft of the article.*

REFERENCES

1. Gallegos A. Physician liability: Your team, your legal risk. American Medical News. Cited 2013, July 29. Available from: http://www.amednews.com/article/20130729/profession/130729942/4/
2. ECRI Institute. Intervention methods improve physician-nurse communication. Risk Management Reporter, 24(3). Cited 2005, June. Available from: https://www.ecri.org/Pages/default.aspx
3. Niv Y. Pathologists and gastroenterologists. J Clin Pathol. 2003; 56(4):241–2.
4. Fleming KA. Evidenced-based cellular pathology. Lancet 2002; 359(9312):1149–50.
5. Lewin DN, Lewin KJ, Weinstein WM. Pathologist-gastroenterologist interaction. The changing role of the pathologist. Am J Clin Pathol. 1995;103(4 Suppl 1):S9-12.
6. Lal N, Bhasin DK, Malik AK, Gupta M, Singh K, Mehta SK. Optimal number of biopsy specimens in the diagnosis of carcinoma of the esophagus. Gut. 1992; 33(6):724–6.
7. Feakins RM, British Society of Gastroenterology. Inflammatory bowel disease biopsies: updated British Society of Gastroenterology reporting guidelines. J Clin Pathol. 2013; 66(12):1005–26.
8. Novak G, Stevens T, Van Viegen T, Bossuyt P, Štabuc B, Jeyarajah J, et al. Evaluation of optimal biopsy location for assessment of histological activity, transcriptomic and immunohistochemical analyses in patients with active Crohn's disease. Aliment Pharmacol Ther. 2019 ;4 9(11):1401–9.
9. Magro F, Gionchetti P, Eliakim R, Ardizzone S, Armuzzi A, Barreiro-de Acosta M, et al. Third European evidence-based consensus on diagnosis and management of ulcerative colitis. Part 1: Definitions, diagnosis, extra-intestinal manifestations, pregnancy, cancer surveillance, surgery, and ileo-anal pouch disorders. J Crohns Colitis. 2017; 11(6):649–70.
10. Yeshi K, Ruscher R, Hunter L, Daly NL, Loukas A, Wangchuk P. Revisiting Inflammatory Bowel Disease: Pathology, Treatments, Challenges and Emerging Therapeutics Including Drug Leads from Natural Products. J Clin Med. 2020; 9(5):1273.
11. C.R. Snyder, Carol E. Ford. Coping with Negative Life Events: Clinical and Social Psychological Perspectives. Springer Science. (2013). p. 12.
12. Genta RM, Lash RH. Every gastroenterologist deserves a gastrointestinal pathologist. Dig Liver Dis. 2008; 40(8):627-31.
13. Koh TJ, Wang TC. Tumors of the stomach. In: Sleisenger and Fordtran's gastrointestinal and liver disease, 7th ed. Philadelphia: Saunders, 2002:829–55.
14. O'Brien MJ, Winawer SJ, Zauber AG, Gottlieb LS, Sternberg SS, Diaz B, et al. The national polyp study: patient and polyp characteristics associated with high-grade dysplasia in colorectal adenomas. Gastroenterology. 1990; 98(2):371–5.
15. Sands BE. Crohn's disease. In: Sleisenger and Fordtran's gastrointestinal and liver disease, 7th ed. Philadelphia: Saunders, 2002:2005–38.
16. Dube AK, Cross SS, Lobo AJ. Audit of the histopathological diagnosis of non-neoplastic colorectal biopsies: achievable standards for the diagnosis of inflammatory bowel disease. J Clin Pathol 1998; 51(5):378–81.
17. Misiewicz J. The Sidney system: a new classification of gastritis. J Gastroenterol Hepatol 1991; 6(3):207–8.
18. Kahrilas PJ, Pandolfino JE. Gastroesophageal reflux disease and its complications, including Barrett's metaplasia. In: Sleisenger and Fordtran's gastrointestinal and liver disease, 7th ed. Philadelphia: Saunders, 2002:599–622.
19. Jewell DP. Ulcerative colitis. In: Sleisenger and Fordtran's gastrointestinal and liver disease, 7th ed. Philadelphia: Saunders, 2002; 2039–67.
20. Attanoos RL, Bull AD, Douglas-Jones AG, Fligelstone LJ, Semararo D. Phraseology in pathology reports. A comparative study of interpretation among pathologists and surgeons.

J Clin Pathol 1996; 49(1):79–81.
21. Schwartz WB, Wolfe HJ, Pauker SG. Pathology and probabilities: a new approach to interpreting and reporting biopies. N Engl J Med 1981; 305(16):917–23.
22. Arkteg CB, Wergeland Sørbye S, Buhl Riis L, Dalen SM, Florholmen J, Goll R. Real-life evaluation of histologic scores for Ulcerative Colitis in remission. PLoS One. 2021: 16(3): e0248224.

Corresponding author:

Evangeli Lampri
Department of Pathology, Faculty of Medicine, School of Health Sciences, University of Ioannina, University Campus, PO Box 1186 45 110, Ioannina, Greece
E-mail: elampri@uoi.gr

Principles for the management of pulmonary embolism: An evidence-based review

Aggeliki Bellou[1], Charalampos Lampropoulos[2], Fotini Fligou[1]

Abstract

Pulmonary embolism (PE) is the third most common cause of cardiovascular death worldwide, affecting people of all ages, nationalities, and genders, with an increased incidence in elderly hospitalized patients. PE may present with a spectrum of clinical manifestations ranging from asymptomatic PE to life-threatening PE with hemodynamic instability. Hemodynamic instability is particularly important because it is associated with the risk of premature death. The management of PE has evolved in recent years with the availability of direct oral anticoagulants (DOACs), local thrombolysis, surgical embolectomy, and extracorporeal membrane oxygenation (ECMO). The increasing awareness of healthcare professionals and the development of multidisciplinary PE response teams have also led to significant changes in disease management. In this review we present the latest updates on the management of PE, taking into account the latest ESC / ERS guidelines published in 2019. In addition, we present the most recent publications regarding the occurrence of VTE in COVID-19 patients, the effect of vaccination against SARS CoV-2 on VTE and the most important studies that have been conducted.

Key words: *Venous thromboembolic disease; Pulmonary embolism; Thrombolysis; Low molecular weight heparin (LMWH); Direct oral anticoagulants (DOACs)*

INTRODUCTION

Pulmonary embolism (PE) is defined as the blockage of the pulmonary circulation by a substance that has moved from elsewhere in the body through the bloodstream (embolism). In most cases, PE is caused by a thrombus or thrombi that originate in the deep venous system of the lower or (less often) upper extremities. Rarely, PE is the result of fat, amniotic fluid, parasites, or even air embolism into the pulmonary circulation. Such cases are also known as non-thrombotic PE [1]. PE and deep vein thrombosis (DVT) are two different entities of a main disease called venous thromboembolic disease (VTE). VTE is the third most common acute cardiovascular disease and is responsible for causing significant morbidity and mortality, as well as for a significant financial burden on health care systems [2,3]. Although the incidence of PE seems to increase over the years, the mortality rate decreases [4]. This is due to the adherence to the guidelines by clinicians, as well as to the implementation of safer and more effective treatments and non-invasive diagnostic techniques that have emerged in recent years [5].

[1]Intensive Care Unit, Department of Anesthesiology and Intensive Care Medicine, University of Patras Medical School, Rio, Patras, Greece
[2]Intensive Care Unit, Saint Andrew's General Hospital, Patras, Greece

Received: 05 Apr 2022; Accepted: 22 Jun 2022

The prognosis of PE depends on the patient's hemodynamic compromise, the underlying disease state, and the accurate and prompt diagnosis and treatment. Approximately 34% of patients with PE die suddenly or within a few hours of the acute event, before receiving appropriate treatment, according to epidemiological models [6]. PE is a challenge for clinicians, not only in terms of correct diagnosis, but also regarding appropriate treatment to be applied during the acute phase and long-term follow-up.

The present review summarizes the latest evidence-based recommendations from the European Society of Cardiology (ESC) and the European Respiratory Society (ERS) regarding acute phase and long-term treatment of PE [7]. Acute phase treatment is determined by risk assessment, while long-term treatment is determined by the risk of recurrence.

Assessment of the severity of PE

Assessing the severity of PE is crucial not only for assessing early mortality but also for determining treatment strategy. The severity of PE can be assessed by evaluating the clinical, imaging, and laboratory biological markers associated with right ventricular dysfunction, as well as by evaluating patient's co-morbidities. Prognostic assessment is important to begin upon suspicion of the disease. There are several clinical scores to determine the severity of PE [8,9]. Most of these are based on clinical findings at the time of diagnosis and risk factors for PE. The Pulmonary Embolism Severity Index (PESI) score is the most frequently used score and has been validated by the ESC / ERS 2019 guidelines [10]. The PESI score evaluates 11 parameters including age, sex, temperature, blood pressure, oxygen saturation, and several co-morbidities. Due to the complexity of the original version, a more simplified version has been developed (Table 1). The main limitation of the PESI score is, as mentioned above, that it includes many variables, which makes this score complex and difficult in everyday clinical practice [11,12].

Hemodynamic instability is of particular importance and reflects the right ventricular compromise, hence the risk of premature death. Hemodynamic instability is a rare phenomenon in acute PE, but when present it requires urgent and appropriate medical care from a multidisciplinary team. Imaging of the right ventricle with either an

Table 1. *Original and simplified PESI scores.*

Original PESI score	Simplified PESI score
Variable - Points	***Variable - Points***
1. Age: +1 per year	1. Age > 80 years: +1
2. Sex: Female: 0, Male: +10	2. History of heart failure or chronic lung disease: +1
3. History of cancer: +30	3. History of cancer: +1
4. History of heart failure: +10	4. Heart rate ≥110/min: +1
5. History of chronic lung disease: +10	5. Systolic BP <100 mmHg: +1
6. Heart rate ≥110/min: +20	6. O2 saturation <90%: +1
7. Systolic BP <100 mmHg: +30	
8. Respiratory rate ≥30/min: +20	
9. Temperature <36°C/96.8°F: +20	
10. Altered mental status (disorientation, lethargy, stupor, or coma): +60	
11. O2 saturation <90%: +20	
Classification	Classification
• Class I (≤ 65 points): very low risk	• 0 points: low risk
• Class II (66-85 points): low risk	• ≥ 1 points: high risk
• Class III (86-105 points): intermediate risk	
• Class IV (106-125 points): high risk	
• Class V (> 125 points): very high risk	

echocardiogram or CTPA is vital to detect changes in the morphology and function of the right heart [13,14]. These changes are the result of an acute increase in pulmonary arterial pressure (PAP) and pulmonary vascular resistance (PVR). Biochemical markers that reflect the damage of the myocardium include high circulating levels of highly sensitive troponine I (hs-TNI), brain natriuretic peptide (BNP) and pro-BNP [15,16]. Among the other clinically applicable biomarkers, high serum creatinine and lactic acid levels have been shown to be associated with adverse outcomes in patients with PE [17,18].

Acute phase treatment

During acute phase treatment, the respiratory and hemodynamic stabilization of the patient with PE is of paramount importance.

Ventilation and oxygen therapy: All patients with an oxygen saturation of less than 90% should receive supplemental oxygen therapy. Low arterial oxygen pressure (pO_2), which does not respond to conventional oxygen therapy, may be present in patients with right-to-left communication (open foramen ovale) [19]. Particular care should be taken when applying positive end-expiratory pressure to patients with PE, given that positive thoracic pressure may reduce venous return and cardiac output due to right ventricular failure. High flow nasal cannula or non-invasive ventilation with low PEEP should be preferred if the patient can tolerate these methods of ventilation. When mechanical ventilation is necessary, tidal volume (Vt) should be approximately 6ml/kg ideal body weight (IBW), and the end expiratory plateau pressure should be below 30cm H_2O.

Right ventricular dysfunction: This condition is the main cause of decreased cardiac output and the leading cause of death in patients at high risk for PE. Fluids should be administered with caution, as they may further reduce cardiac output [20]. Assessment of central venous pressure (CVP) can help assess a patient's volume status, especially in hypovolemic patients. Vasoconstrictors and inotropes are often necessary, along with other medication [21]. Norepinephrine ameliorates hemodynamic parameters, improving heart contraction and coronary perfusion, without altering pulmonary vascular resistance (PVR). Dobutamine can be used in patients with normal arterial blood pressure, but physicians should be aware that when used without vasoconstrictors it may aggravate or trigger arrhythmias.

Extracorporeal membrane oxygenation (ECMO): The use of venous-arterial ECMO in patients with PE and hemodynamic compromise is controversial and, when necessary, it requires additional intervention, such as surgical embolectomy. Attention should be given to long term application, due to the complications that may occur (hemorrhage) [22]. The efficacy and safety of ECMO depends on the center's experience and the available expertise [23].

Advanced Life Support (ALS): All healthcare professionals should have the practical skills to manage cardiac arrest and "peri-arrest" problems, according to the European Resuscitation Council (ERC) guidelines [24].

Initial Anticoagulation: Vitamin K antagonists (VKAs) were the mainstay of VTE treatment for more than 50 years. With the introduction of direct oral anticoagulants (DOACs) in the last decade, the therapeutic management of PE has undergone radical changes. However, parenteral anticoagulants (low molecular weight heparin - LMWH, fondaparinux and unfractionated heparin - UFH) still remain the mainstay of treatment for initial anticoagulant therapy in VTE (Table 2).

Anticoagulants: As mentioned above, the treatment of acute phase PE is based on risk assessment. In patients with high-risk PE, it is strongly recommended to start anticoagulant therapy with UFH, including a weight-adjusted bolus dose. In patients with intermediate or low-risk PE, immediate initiation of anticoagulant therapy with LMWH is recommended, when the pre-test probability is intermediate or high. LMWH and fondaparinux are preferred because of lower risk of major hemorrhage or heparin-induced thrombocytopenia (HIT) compared to UFH [25,26]. The use of UFH is limited to patients with hemodynamic compromise who may require reperfusion intervention or to patients with mechanical heart valves and severe renal insufficiency.

An equally fast anticoagulant effect is achieved with VKAs and DOACs. DOACs are molecules that directly inhibit several coagulation factors. Specifically, dabi-

Table 2. *Parenteral anticoagulants.*

Agent	Dosage	Interval
Fondaparinux	5mg (body weight <50kg) 7.5mg (body weight 50-100kg) 10mg (body weight >100kg)	Once daily
Enoxaparin	1mg/kg	Twice daily
Tinzaparin	175 IU/kg	Once daily
Dalteparin	100 IU/kg	Twice daily

gatran inhibits thrombin, while edoxaban, rivaroxaban and apixaban inhibit factor Xa (Table 3). In several studies apixaban has been shown to be safer for major or clinically relevant non-major bleeding [27]. DOACs are not recommended for patients with severe renal impairment, antiphospholipid syndrome, or patients who are pregnant or breastfeeding. When VKAs are used, concomitant parenteral anticoagulant therapy is recommended for at least 5 days, until INR reaches 2-3 for two consecutive days. Warfarin should be initiated in patients under 60 years old at a dose of 10 mg, while in the elderly at a dose of 5 mg. The dose should then be adjusted over the next 5-7 days, according to INR levels. VKAs have many limitations, especially regarding the need for frequent INR measurements and their pharmacokinetics when taking other medications or foods [28].

Reperfusion treatment: Patients with PE who are hemodynamically unstable should undergo thrombolysis in an intensive care unit. Thrombolysis has been shown to reduce PAP, PVR and RV dilatation. Thrombolysis is most effective when performed within the first 48 hours of the onset of symptoms, although it may be useful in patients with symptom onset for up to 14 days [29]. Thrombolysis is considered successful when the patient's hemodynamic status and RV dysfunction improve (as shown on the echocardiography 36 hours after thrombolysis) [30]. The impact of thrombolysis has been studied in the Pulmonary Embolism Thrombolysis Study (PEITHO trial) where patients with intermediate high pulmonary embolism underwent thrombolysis. The researchers concluded that although thrombolysis significantly improved RV dysfunction and resulted in reduced mortality from haemodynamic collapse, it increased the risk of severe bleeding [31]. The approved regimens for thrombolysis and contraindications are shown in Table 4 and Table 5, respectively.

Alternatively, patients with high-risk PE or patients with intermediate high PE who worsen may undergo mechanical reperfusion. This is performed by a percutaneous catheter which is directed to the pulmonary arterial bed and fragments and/or aspirates the thrombus. A hybrid method that combines mechanical fragmentation of the thrombus with in situ low dose thrombolysis may also be performed. The overall success rate of percutaneous catheter-directed therapy has been mentioned to be up to 87% [32]. Surgical embolectomy in high-risk PE is performed along with cardiopulmonary bypass and seeks to aspirate fresh thrombi. Recent studies support surgical embolization in combination with ECMO in patients with high-risk PE [33].

Table 3. *Direct oral anticoagulants (DOACs).*

Agent	Dosage
Dabigatran*	150mg twice daily
Rivaroxaban	15mg twice daily for 21 days, followed by 20mg once daily
Apixaban	10mg twice daily for 7 days, followed by 5mg twice daily
Edoxaban *	60mg once daily

*It should always be preceded by parenteral anticoagulants for at least 5 days.

Vena cava filters: The placement of a vena cava filter is intended to mechanically prevent thrombi displacement from the lower extremities into the pulmonary circulation. Most vena cava filters are placed percutaneously and can be removed after weeks or months, or left in place for a long time, if necessary. According to the 2019 ESC/ERS guidelines, the indications for placement

Table 4. *Thrombolytic regiments.*

Agent	Dosage
rtPA (alteplase)	100mg over 2 hrs
Streptokinase	250000 IU over 30 min as a loading dose, followed by 100000 IU/hr over 12-24 hrs
Urokinase	4400 IU/kg over 10 min as a loading dose, followed by 4400 IU/kg/hr over 12-24 hrs

Table 5. *Contraindications to thrombolysis.*

Absolute	Relative
Prior intracranial haemorrhage	Transient ischaemic attack within 6 months
Known intracranial neoplasm	Oral anticoagulations
Ischaemic stroke within 6 months	Pregnancy or post-partum week
Major trauma or head injury in previous 3 weeks	Active peptic ulcer
Bleeding diathesis	Uncontrolled hypertension (SBP>180mmHg)
Active bleeding (except menses)	Non-compressible puncture sites
	Advanced liver disease
	Traumatic resuscitation

of a vena cava filter include the following: a) absolute contraindications for anticoagulation, and b) recurrent VTE despite adequate anticoagulation therapy. The PREPIC-2 study showed that vena cava filters compared to standard anticoagulant treatment were associated with a lower risk of PE recurrence, but a significantly higher risk of DVT, and no statistically significant difference in mortality risk [34,35].

Long-term treatment

Long term treatment of VTE aims to: a) be completed without complications, and b) prevent relapses. Most studies on the long-term treatment of VTE include patients with DVT, with or without PE. The risk for recurrence after discontinuation of treatment is associated with the characteristics of the principal event [36]. The recurrence rate after discontinuation of treatment is approximately 2.5% per year for PE associated with transient risk factors, compared to approximately 4.5% per year for PE occurring in the absence of known malignancy, thrombophilia, or other known risk factors [37,38]. The recurrence rate after discontinuation of anticoagulant therapy is similar whether it will stop after 3-6 months or after longer time periods. In addition, it should be borne in mind that although per se anticoagulant therapy reduces the risk of VTE by approximately 90%, it also increases the risk of bleeding (Table 6) [39,40,41].

According to recent guidelines, all patients with VTE should receive anticoagulant therapy for at least 3 months [42]. For patients in whom the first episode of VTE is the result of a major transient or reversible risk factor, discontinuation of anticoagulant therapy at 3 months is recommended [43]. For patients in whom VTE is not associated with a major or reversible risk factor, an indefinite duration of treatment is recommended [44]. VKAs are the treatment of choice in patients with antiphospholipid syndrome [45]. Patients with hereditary thrombophilia, especially those with confirmed protein C, S, antithrombin deficiency or homozygous mutation in prothrombin G20210A, are candidates for indefinite duration of treatment when the main VTE event occurs in the absence of a major transient or reversible risk factor. However, there are no data on the clinical benefits of prolonged anticoagulant therapy in carriers of the G20210A mutation or in patients with heterozygous factor V Leiden. An indefinite duration of treatment is recommended for the first episode of VTE, when there is not known risk factor. In prolonged treatment, DOACs could be used at a reduced dose. In prolonged treatment with DOACs reduced doses may be used [46,47]. Patient compliance, hepatic and renal function should be assessed on a regular basis.

PE during pregnancy

Pulmonary embolism is one of the leading causes of maternal death during pregnancy and treatment must be accurate and immediate [48,49]. According to recent guidelines, any pregnant woman with high or intermediate/low pre-test probability and positive d-dimmer levels should receive LMWH anticoagulant treatment without delay. UFH is not a contraindication during pregnancy, especially when the patient is hemodynamically unstable. UFH should be stopped 6 hours before the delivery [50]. VKAs and DOACs are contraindicated during pregnancy, as they cross the placenta [51]. ESC guidelines recommend thrombolysis in pregnant patients with PE who deteriorate hemodynamically. Health professionals should always keep in mind that PE during pregnancy should be treated by a multidisciplinary team (PERT), including obstetricians.

PE in patients with a malignancy

Patients with a malignancy are at greater risk of developing VTE. In addition, it is widely accepted that patients with a malignancy have a higher recurrence rate of VTE under LMWH (7-9%) compared to patients without malignancy [52]. 2019 ESC / ERS guidelines recommend that patients with a malignancy should receive LMWH for at least 6 months [53,54]. Nowadays, DOACs have been studied in patients with a malignancy. Rivaroxaban and edoxaban may be used in such patients with a malignancy, unless they suffer from gastrointestinal malignancy [55,56]. Patients with a malignancy

Table 6. *Patients at increased risk of bleeding.*

1. Bleeding within last 30 days needing acute care setting
2. Bleeding disorders
3. Central nervous system malignancy
4. Thrombolysis within 7 days
5. Oral anticoagulants
6. History of intracranial haemorrhage
7. Recent ischemic stroke
8. Platelet count < 50 x 10^9/L, Haeboglobin < 8 g/dL
9. Major surgery within 14 days

diagnosed with PE incidentally (for instance, during follow-up CT) should be treated in the same way as symptomatic patients, even if PE involves a single subsegmental vessel [57]. There is no current data to support the use of vena cava filters as an adjunct to anticoagulant therapy in this group of patients.

PE and COVID-19 infection

Early in the course of COVID-19 disease it was observed that inpatients develop a pro-coagulant state (eg. elevated d-dimers, high levels of von Willebrand and VII factor, platelet activation), leading not only to macrovascular but also microvascular in situ thrombosis [58,59]. This finding has led many researchers to seek the optimal treatment for both thromboprophylaxis and therapeutic anticoagulant regimen in the short and long term. According to the guidelines published in CHEST in February 2022, it is recommended to administer a therapeutic dose (as thromboprophylaxis) of UFH or LMWH (preferably LMWH to reduce staff exposure) in acutely ill patients, that have low bleeding risk. On the other hand, for critically ill patients it is recommended to administer a prophylactic dose, instead of the intermediate or therapeutic dose [60]. Large studies have shown that heparin administered in the early stages of the disease has antiviral and anti-inflammatory action, which are absent in severe ARDS from COVID-19 [61]. In addition, ICU patients were more likely to experience major bleeding, and there was no difference in mortality rates compared with those receiving the therapeutic or intermediate dose of UFH or LMWH [62]. DOACs have no indication for acute thromboprophylaxis or treatment of VTE in those patients [63]. Anticoagulant therapy is not recommended in COVID-19 positive patients who do not require hospitalization [64]. Continuation of heparin is not recommended after discharge [65]. Regarding long term treatment, there are no clear data or large randomized studies. However, it is a fact that the majority of complications occur in the first 30 days. Current guidelines fail to clarify the optimal length of time that anticoagulants will be necessary for patients with COVID-19 and VTE, and it is currently suggested that treatment is similar to standard of care patients.

PE and vaccination against SARS CoV-2

Vaccination against SARS CoV-2 is the most important strategy for ending the pandemic of the disease. Currently in the medical quiver there are several vaccines with different modes of action and different effectiveness [66]. All vaccines are generally safe and effective, and they do not appear to cause more VTE events compared to SARS CoV-2 infection, even in specific patient subgroups [67,68]. There are no robust data to support the exception from vaccination of patients with thrombophilia or to use thromboprophylaxis for a certain time period [69].

CONCLUSIONS

Optimal management of PE involves a multidisciplinary approach and treatment should be individualized. Patient management should start upon disease suspicion using validated risk stratification algorithms. LMWHs remain the treatment of choice for initial anticoagulant therapy. Over the past decade, four DOACs have led to significant changes not only in the chronic treatment of PE but also in the initial anticoagulant therapy. These agents have been tested and proven to be safe for patients with malignancies other than gastrointestinal malignancies. Thrombolysis should be performed in an intensive care unit, with monitoring, and always taking into account absolute contraindications. There is still controversy over the optimal dose of heparin during pregnancy; DOACs are not recommended during pregnancy. Further research is needed to determine the ideal period for anticoagulant therapy based on the risk of recurrence.

Conflict of interest disclosure: *None to declare.*

Declaration of funding sources: *None to declare*

Author Contributions: *A. Bellou, study conception and design, analysis and interpretation of data; A. Bellou and Ch. Lampropoulos, drafting of manuscript; F. Fligou, critical revision of manuscript.*

REFERENCES

1 Jorens PG, Van Marck E, Snoeckx A, Parizel PM. Nonthrombotic pulmonary embolism. Eur Respir J. 2009;34(2):452-74.
2 Wendelboe AM, Raskob GE. Global Burden of Thrombosis: Epidemiologic Aspects. Circ Res. 2016;118(9):1340-7.
3 Barco S, Woersching AL, Spyropoulos AC, Piovella F, Mahan CE. European Union-28: An annualised cost-of-illness model for venous thromboembolism. Thromb Haemost. 2016;115(4):800-8.
4 Jiménez D, de Miguel-Díez J, Guijarro R, Trujillo-Santos J, Otero R, Barba R, et al. Trends in the Management and Outcomes of Acute Pulmonary Embolism: Analysis From the RIETE Registry. J Am Coll Cardiol. 2016;67(2):162-70.

5 Jiménez D, Bikdeli B, Barrios D, Morillo R, Nieto R, Guerassimova I, et al. Management appropriateness and outcomes of patients with acute pulmonary embolism. Eur Respir J. 2018;51(5):1800445.
6 Cohen AT, Agnelli G, Anderson FA, Arcelus JI, Bergqvist D, Brecht JG, et al. Venous thromboembolism (VTE) in Europe. The number of VTE events and associated morbidity and mortality. Thromb Haemost. 2007;98(4):756-64.
7 Konstantinides SV, Meyer G, Becattini C, Bueno H, Geersing GJ, Harjola VP, et al. 2019 ESC Guidelines for the diagnosis and management of acute pulmonary embolism developed in collaboration with the European Respiratory Society (ERS). Eur Heart J. 2020;41(4):543-603.
8 Dellas C, Tschepe M, Seeber V, Zwiener I, Kuhnert K, Schäfer K, et al. A novel H-FABP assay and a fast prognostic score for risk assessment of normotensive pulmonary embolism. Thromb Haemost. 2014;111(5):996-1003.
9 Lankeit M, Friesen D, Schäfer K, Hasenfuß G, Konstantinides S, Dellas C. A simple score for rapid risk assessment of non-high-risk pulmonary embolism. Clin Res Cardiol. 2013;102(1):73-80.
10 Donzé J, Le Gal G, Fine MJ, Roy PM, Sanchez O, Verschuren F, et al. Prospective validation of the Pulmonary Embolism Severity Index. A clinical prognostic model for pulmonary embolism. Thromb Haemost. 2008;100(5):943-8.
11 Jiménez D, Aujesky D, Moores L, Gómez V, Lobo JL, Uresandi F, et al. Simplification of the pulmonary embolism severity index for prognostication in patients with acute symptomatic pulmonary embolism. Arch Intern Med. 2010;170(15):1383-9.
12 Sam A, Sánchez D, Gómez V, Wagner C, Kopecna D, Zamarro C, et al. The shock index and the simplified PESI for identification of low-risk patients with acute pulmonary embolism. Eur Respir J. 2011;37(4):762-6.
13 Pruszczyk P, Goliszek S, Lichodziejewska B, Kostrubiec M, Ciurzyński M, Kurnicka K, et al. Prognostic value of echocardiography in normotensive patients with acute pulmonary embolism. JACC Cardiovasc Imaging. 2014;7(6):553-60.
14 Aviram G, Soikher E, Bendet A, Shmueli H, Ziv-Baran T, Amitai Y, et al. Prediction of Mortality in Pulmonary Embolism Based on Left Atrial Volume Measured on CT Pulmonary Angiography. Chest. 2016;149(3):667-75.
15 Lankeit M, Friesen D, Aschoff J, Dellas C, Hasenfuss G, Katus H, et al. Highly sensitive troponin T assay in normotensive patients with acute pulmonary embolism. Eur Heart J. 2010;31(15):1836-44.
16 Klok FA, Mos IC, Huisman MV. Brain-type natriuretic peptide levels in the prediction of adverse outcome in patients with pulmonary embolism: a systematic review and meta-analysis. Am J Respir Crit Care Med. 2008;178(4):425-30.
17 Kostrubiec M, Pływaczewska M, Jiménez D, Lankeit M, Ciurzynski M, Konstantinides S, et al. The Prognostic Value of Renal Function in Acute Pulmonary Embolism-A Multi-Centre Cohort Study. Thromb Haemost. 2019;119(1):140-8.
18 Vanni S, Viviani G, Baioni M, Pepe G, Nazerian P, Socci F, et al. Prognostic value of plasma lactate levels among patients with acute pulmonary embolism: the thrombo-embolism lactate outcome study. Ann Emerg Med. 2013;61(3):330-8.
19 Konstantinides S, Geibel A, Kasper W, Olschewski M, Blümel L, Just H. Patent foramen ovale is an important predictor of adverse outcome in patients with major pulmonary embolism. Circulation. 1998;97(19):1946-51.
20 Mercat A, Diehl JL, Meyer G, Teboul JL, Sors H. Hemodynamic effects of fluid loading in acute massive pulmonary embolism. Crit Care Med. 1999;27(3):540-4.
21 Ventetuolo CE, Klinger JR. Management of acute right ventricular failure in the intensive care unit. Ann Am Thorac Soc. 2014;11(5):811-22.
22 Corsi F, Lebreton G, Bréchot N, Hekimian G, Nieszkowska A, Trouillet JL, et al. Life-threatening massive pulmonary embolism rescued by venoarterial-extracorporeal membrane oxygenation. Crit Care. 2017;21(1):76.
23 Al-Bawardy R, Rosenfield K, Borges J, Young MN, Albaghdadi M, Rosovsky R, et al. Extracorporeal membrane oxygenation in acute massive pulmonary embolism: a case series and review of the literature. Perfusion. 2019;34(1):22-8.
24 Soar J, Böttiger BW, Carli P, Couper K, Deakin CD, Djärv T, et al. European Resuscitation Council Guidelines 2021: Adult advanced life support. Resuscitation. 2021;161:115-51.
25 Cossette B, Pelletier ME, Carrier N, Turgeon M, Leclair C, Charron P, et al. Evaluation of bleeding risk in patients exposed to therapeutic unfractionated or low-molecular-weight heparin: a cohort study in the context of a quality improvement initiative. Ann Pharmacother. 2010;44(6):994-1002.
26 van Den Belt AG, Prins MH, Lensing AW, Castro AA, Clark OA, Atallah AN, et al. Fixed dose subcutaneous low molecular weight heparins versus adjusted dose unfractionated heparin for venous thromboembolism. Cochrane Database Syst Rev. 2000;(2):CD001100.
27 Cohen AT, Hamilton M, Mitchell SA, Phatak H, Liu X, Bird A, et al. Comparison of the Novel Oral Anticoagulants Apixaban, Dabigatran, Edoxaban, and Rivaroxaban in the Initial and Long-Term Treatment and Prevention of Venous Thromboembolism: Systematic Review and Network Meta-Analysis. PLoS One. 2015;10(12):e0144856.
28 Witt DM, Clark NP, Kaatz S, Schnurr T, Ansell JE. Guidance for the practical management of warfarin therapy in the treatment of venous thromboembolism. J Thromb Thrombolysis. 2016;41(1):187-205.
29 Daniels LB, Parker JA, Patel SR, Grodstein F, Goldhaber SZ. Relation of duration of symptoms with response to thrombolytic therapy in pulmonary embolism. Am J Cardiol. 1997;80(2):184-8.
30 Martin C, Sobolewski K, Bridgeman P, Boutsikaris D. Systemic Thrombolysis for Pulmonary Embolism: A Review. P T. 2016;41(12):770-5.
31 Meyer G, Vicaut E, Danays T, Agnelli G, Becattini C, Beyer-Westendorf J, et al. Fibrinolysis for patients with intermediate-risk pulmonary embolism. N Engl J Med. 2014;370(15):1402-11.
32 Bajaj NS, Kalra R, Arora P, Ather S, Guichard JL, Lancaster WJ, et al. Catheter-directed treatment for acute pulmonary

embolism: Systematic review and single-arm meta-analyses. Int J Cardiol. 2016;225:128-39.
33 Wu MY, Liu YC, Tseng YH, Chang YS, Lin PJ, Wu TI. Pulmonary embolectomy in high-risk acute pulmonary embolism: the effectiveness of a comprehensive therapeutic algorithm including extracorporeal life support. Resuscitation. 2013;84(10):1365-70.
34 Mismetti P, Laporte S, Pellerin O, Ennezat PV, Couturaud F, Elias A, et al. Effect of a retrievable inferior vena cava filter plus anticoagulation vs anticoagulation alone on risk of recurrent pulmonary embolism: a randomized clinical trial. JAMA. 2015;313(16):1627-35.
35 Eight-year follow-up of patients with permanent vena cava filters in the prevention of pulmonary embolism: the PREPIC (Prevention du Risque d'Embolie Pulmonaire par Interruption Cave) randomized study. Circulation. 2005;112(3):416-22.
36 Murin S, Romano PS, White RH. Comparison of outcomes after hospitalization for deep venous thrombosis or pulmonary embolism. Thromb Haemost. 2002;88(3):407-14.
37 Schulman S, Rhedin AS, Lindmarker P, Carlsson A, Lärfars G, Nicol P, et al. A comparison of six weeks with six months of oral anticoagulant therapy after a first episode of venous thromboembolism. Duration of Anticoagulation Trial Study Group. N Engl J Med. 1995;332(25):1661-5.
38 Kearon C, Ageno W, Cannegieter SC, Cosmi B, Geersing GJ, Kyrle PA. Categorization of patients as having provoked or unprovoked venous thromboembolism: guidance from the SSC of ISTH. J Thromb Haemost. 2016;14(7):1480-3.
39 Couturaud F, Sanchez O, Pernod G, Mismetti P, Jego P, Duhamel E, et al. Six Months vs Extended Oral Anticoagulation After a First Episode of Pulmonary Embolism: The PADIS-PE Randomized Clinical Trial. JAMA. 2015;314(1):31-40.
40 Campbell IA, Bentley DP, Prescott RJ, Routledge PA, Shetty HG, Williamson IJ. Anticoagulation for three versus six months in patients with deep vein thrombosis or pulmonary embolism, or both: randomised trial. BMJ. 2007;334(7595):674.
41 Linkins LA, Choi PT, Douketis JD. Clinical impact of bleeding in patients taking oral anticoagulant therapy for venous thromboembolism: a meta-analysis. Ann Intern Med. 2003;139(11):893-900.
42 Boutitie F, Pinede L, Schulman S, Agnelli G, Raskob G, Julian J, et al. Influence of preceding length of anticoagulant treatment and initial presentation of venous thromboembolism on risk of recurrence after stopping treatment: analysis of individual participants' data from seven trials. BMJ. 2011;342:d3036.
43 Baglin T, Luddington R, Brown K, Baglin C. Incidence of recurrent venous thromboembolism in relation to clinical and thrombophilic risk factors: prospective cohort study. Lancet. 2003;362(9383):523-6.
44 Schulman S, Granqvist S, Holmström M, Carlsson A, Lindmarker P, Nicol P, et al. The duration of oral anticoagulant therapy after a second episode of venous thromboembolism. The Duration of Anticoagulation Trial Study Group. N Engl J Med. 1997;336(6):393-8.
45 Schulman S, Svenungsson E, Granqvist S. Anticardiolipin antibodies predict early recurrence of thromboembolism and death among patients with venous thromboembolism following anticoagulant therapy. Duration of Anticoagulation Study Group. Am J Med. 1998;104(4):332-8.
46 Weitz JI, Lensing AWA, Prins MH, Bauersachs R, Beyer-Westendorf J, Bounameaux H, et al. Rivaroxaban or Aspirin for Extended Treatment of Venous Thromboembolism. N Engl J Med. 2017;376(13):1211-22.
47 Agnelli G, Buller HR, Cohen A, Curto M, Gallus AS, Johnson M, et al. Apixaban for extended treatment of venous thromboembolism. N Engl J Med. 2013;368(8):699-708.
48 Creanga AA, Syverson C, Seed K, Callaghan WM. Pregnancy-Related Mortality in the United States, 2011-2013. Obstet Gynecol. 2017;130(2):366-73.
49 Henriksson P, Westerlund E, Wallén H, Brandt L, Hovatta O, Ekbom A. Incidence of pulmonary and venous thromboembolism in pregnancies after in vitro fertilisation: cross sectional study. BMJ. 2013;346:e8632.
50 Leffert L, Butwick A, Carvalho B, Arendt K, Bates SM, Friedman A, et al. The Society for Obstetric Anesthesia and Perinatology Consensus Statement on the Anesthetic Management of Pregnant and Postpartum Women Receiving Thromboprophylaxis or Higher Dose Anticoagulants. Anesth Analg. 2018;126(3):928-44.
51 Regitz-Zagrosek V, Roos-Hesselink JW, Bauersachs J, Blomstrom-Lundqvist C, Cifkova R, De Bonis M, et al. 2018 ESC Guidelines for the management of cardiovascular diseases during pregnancy. Kardiol Pol. 2019;77(3):245-326.
52 Louzada ML, Carrier M, Lazo-Langner A, Dao V, Kovacs MJ, Ramsay TO, et al. Development of a clinical prediction rule for risk stratification of recurrent venous thromboembolism in patients with cancer-associated venous thromboembolism. Circulation. 2012;126(4):448-54.
53 Meyer G, Marjanovic Z, Valcke J, Lorcerie B, Gruel Y, Solal-Celigny P, et al. Comparison of low-molecular-weight heparin and warfarin for the secondary prevention of venous thromboembolism in patients with cancer: a randomized controlled study. Arch Intern Med. 2002;162(15):1729-35.
54 Lee AY, Levine MN, Baker RI, Bowden C, Kakkar AK, Prins M, et al. Low-molecular-weight heparin versus a coumarin for the prevention of recurrent venous thromboembolism in patients with cancer. N Engl J Med. 2003;349(2):146-53.
55 Raskob GE, van Es N, Verhamme P, Carrier M, Di Nisio M, Garcia D, et al. Edoxaban for the Treatment of Cancer-Associated Venous Thromboembolism. N Engl J Med. 2018;378(7):615-24.
56 Young AM, Marshall A, Thirlwall J, Chapman O, Lokare A, Hill C, et al. Comparison of an Oral Factor Xa Inhibitor With Low Molecular Weight Heparin in Patients With Cancer With Venous Thromboembolism: Results of a Randomized Trial (SELECT-D). J Clin Oncol. 2018;36(20):2017-23.
57 den Exter PL, Hooijer J, Dekkers OM, Huisman MV. Risk of recurrent venous thromboembolism and mortality in patients with cancer incidentally diagnosed with pulmonary

embolism: a comparison with symptomatic patients. J Clin Oncol. 2011;29(17):2405-9.
58 Scudiero F, Silverio A, Di Maio M, Russo V, Citro R, Personeniet D, al. Pulmonary embolism in COVID-19 patients: prevalence, predictors and clinical outcome. Thromb Res. 2021;198:34-9.
59 Poor HD. Pulmonary Thrombosis and Thromboembolism in COVID-19. Chest. 2021;160(4):1471-80.
60 Moores LK, Tritschler T, Brosnahan S, Carrier M, Collen JF, Doerschuget K, al. Thromboprophylaxis in Patients With COVID-19: A Brief Update to the CHEST Guideline and Expert Panel Report. Chest. 2022;162(1):213-25.
61 Hippensteel JA, LaRiviere WB, Colbert JF, Langouët-Astrié CJ, Schmidt EP. Heparin as a therapy for COVID-19: current evidence and future possibilities. Am J Physiol Lung Cell Mol Physiol. 2020;319(2):L211-7.
62 Inspiration Investigators, Sadeghipour P, Talasaz AH, Rashidi F, Sharif-Kashani B, Beigmohammadi MT, et al. Effect of Intermediate-Dose vs Standard-Dose Prophylactic Anticoagulation on Thrombotic Events, Extracorporeal Membrane Oxygenation Treatment, or Mortality Among Patients With COVID-19 Admitted to the Intensive Care Unit: The INSPIRATION Randomized Clinical Trial. JAMA. 2021;325(16):1620-30.
63 Lopes RD, de Barros E Silva PGM, Furtado RHM, Bronhara B, Damianiet LP, al. Therapeutic versus prophylactic anticoagulation for patients admitted to hospital with COVID-19 and elevated D-dimer concentration (ACTION): an open-label, multicentre, randomised, controlled trial. Lancet. 2021;397(10291):2253-63.
64 Cuker A, Tseng EK, Nieuwlaat R, Angchaisuksiri P, Blair C, Dane K, et al. American Society of Hematology living guidelines on the use of anticoagulation for thromboprophylaxis in patients with COVID-19: January 2022 update on the use of therapeutic-intensity anticoagulation in acutely ill patients. Blood Adv. 2022; bloodadvances.2022007561.
65 Roberts LN, Whyte MB, Georgiou L, Giron G, Czuprynska J, Rea C, et al. Postdischarge venous thromboembolism following hospital admission with COVID-19. Blood. 2020;136(11):1347-50.
66 Fiolet T, Kherabi Y, MacDonald CJ, Ghosn J, Peiffer-Smadja N. Comparing COVID-19 vaccines for their characteristics, efficacy and effectiveness against SARS-CoV-2 and variants of concern: a narrative review. Clin Microbiol Infect. 2022;28(2):202-21.
67 Klein NP, Lewis N, Goddard K, Fireman B, Zerbo O, Hansonet KE, al. Surveillance for Adverse Events After COVID-19 mRNA Vaccination. JAMA. 2021;326(14):1390-9.
68 Jabagi MJ, Botton J, Bertrand M, Weill A, Farrington P, Zureiket M, al. Myocardial Infarction, Stroke, and Pulmonary Embolism After BNT162b2 mRNA COVID-19 Vaccine in People Aged 75 Years or Older. JAMA. 2022;327(1):80-2.
69 Bilotta C, Perrone G, Adelfio V, Spatola GF, Uzzo ML, Argo A, et al. COVID-19 Vaccine-Related Thrombosis: A Systematic Review and Exploratory Analysis. Front Immunol. 2021;12:729251.

Corresponding author:

Aggeliki Bellou,
Naxou 4, Rio - Patras, 26504, Greece
E-mail: angelabellou@gmail.com

Emerging molecular targets in Metastatic Colorectal Cancer

Foteinos-Ioannis Dimitrakopoulos, Angelina Bania*, Gerasimia-Marina Chardalia*, Maria Ioakeim*, Athina Katsigianni*, Mari-Liz Knouni*, George C. Kyriakopoulos*, Efthymia Mesiri*, Ioannis A. Pyrousis*, Iasonas Theodorou*, Helen Tsaroucha*, George Tziolis*, Angelos Koutras

Abstract

Colorectal cancer (CRC) is one of the most frequent cancers in both men and women with an increasing incidence in young adults. Despite the expansion of our understanding regarding the biology and pathogenesis, the advances in the treatment of metastatic CRC disease remain limited. Over the past years, a growing number of molecular targets have attracted the interest of the scientific community. Until now, clinical utility has been confirmed for a numerous of these actionable targets. So, new treatment approaches have focused on angiogenesis and immunotherapy as well as novel inhibitors have been developed against EGFR (Epidermal Growth Factor Receptor), KRAS (KRAS Proto-Oncogene, GTPase), BRAF (B-Raf Proto-Oncogene, Serine/Threonine Kinase), HER2 (Erb-B2 Receptor Tyrosine Kinase 2), NTRK (Neurotrophic Receptor Tyrosine Kinase) and others. In this review, we summarize current knowledge on the validated as well as emerging molecular targets in the treatment of metastatic CRC.

Key words: *Colorectal cancer; targets; treatment*

INTRODUCTION

Colorectal cancer (CRC) appears to be the third most commonly diagnosed cancer and one of the predominant causes of cancer-related mortality worldwide, ranking second following lung malignancies. According to the 2020 GLOBOCAN statistics, there have been recorded approximately more than 1.9 million new cases of colorectal cancer (9.8 % of all cancer cases) along with 935,000 deaths (9,2% of total cancer related deaths.), affecting both men and women [1]. Similarly, the American Cancer Society expects 104,270 new cases of colon cancer and 45,230 new cases of rectal cancer, by the end of 2021 in the United States [2].

The incidence of this cancer type has been increasing accordingly to the human development index with the European countries, Australia and Northern America ranking first in the list. This phenomenon has been related to western-type dietary patterns and lifestyle factors, such as smoking and excess meat consumption [3]. But despite the fact that the survival rate has been improving through the years, especially due to the widely used screening methods, changes in certain daily habits but also the evolution of therapeutic strategies, the overall 5-year survival rate, which mainly depends on the stage of the disease, remains at 65-70% in localized and regional stage cancers but drops significantly below 20% for those whose cancer has spread to distant parts of the body (14% for colon cancer and 16% for rectal cancer according to the American Cancer Society's (ACS) data from 2010-2016.

Division of Oncology, Department of Internal Medicine, Medical School, University of Patras, Patras, Greece

* These authors contributed equally to this study

Received: 16 Jan 2022; Accepted: 18 May 2022

Until now, the cornerstone in the treatment of early-stage CRC is the surgical resection of the tumor. However, surgery is rarely a curative option for almost 20-25% of patients that present initially with metastatic cancer or develop later metastatic disease. In these cases, radiotherapy, chemotherapy and most recently immunotherapy are implemented as treatment options.

The heterogeneity of CRC, regarding especially the distinct molecular profile that each tumor has, and therefore its unique clinical features, has generated the necessity to seek alternative treatments. Focusing on molecular targets, these therapeutic strategies are mainly associated with predictive biomarkers, such as microsatellite instability (MSI), mutations in *RAS* (RAS Proto-Oncogene, GTPase) and *BRAF* (B-Raf Proto-Oncogene, Serine/Threonine Kinase) genes, amplification of *HER2* (Erb-B2 Receptor Tyrosine Kinase 2) as well as *NTRK* (Neurotrophic Receptor Tyrosine Kinase) gene fusions and aim to achieve a prolonged survival rate for patients with CRC with significantly less side effects than chemotherapy [4]. An alternative modern treatment option of metastatic CRC is the administration of immunotherapy and especially the immune checkpoint inhibitors which aim to reactivate the immune system response against cancer [5]. In this review, we present current knowledge regarding the molecular targets in the treatment of CRC.

Significant signaling pathways in colorectal cancer

The signaling pathway of the EGFR (epidermal growth factor receptor) has a central role in CRC. The activation of EGFR triggers the activation of PI3K (Phosphoinositide-3-kinase) and MAPK (Mitogen-Activated Protein Kinase) signaling pathways, which constitute significant pathways for cell proliferation, growth and apoptosis inhibition [6,7]. EGFR is present on the cell membranes, while elevated expression levels can be found in neoplastic cells and moderate adenomas [8]. Studies have shown that 60-80% of colorectal tumors have overexpressed EGFR [9].

More specifically, EGFR, a member of the ErbB family of receptor tyrosine kinase, is a transmembrane glycoprotein with an intracellular domain functioning as a tyrosine kinase and an extracellular ligand-binding domain [10]. The ErbB family consists of four ErbB members: ErbB1 (EGFR/HER1), ErbB2 (Neu/HER2), ErbB3 (HER3), and ErbB4 (HER4) [11]. After the ligand binding on the receptor, homo- or hetero-dimerization occurs, phosphorylation of the tyrosine kinase domains is triggered, and the MAPK cascade is activated [10]. Then, the next step of the signaling cascade is the activation of RAS protein. There are three isoforms of Ras GTPases (Guanosine triphosphate) including H-Ras, N-Ras, and K-Ras [12,13].

The RAS protein has two forms, the active GTP bound state, and the GDP (Guanosine diphosphate) bound state [14]. RAF activation by phosphorylation is mediated by active RAS leading to MEK/MAPK (Mitogen-activated protein kinase) activation as well as to phosphorylation and activation of ERK (extracellular signal-related kinase) [15–17]. Phosphorylated ERK translocates from the cytoplasm to the nucleus, as a transcription factor, phosphorylates and regulates various other transcription factors, including carbamoyl phosphate synthetase II (CPS-II) and p90RSK. The final result is the expression of target genes (e.g. *c-FOS, c-JUN and myc*) by the transcription factors, leading to cell survival and growth [18,19].

Role of angiogenesis in colorectal cancer

Angiogenesis is the process through which new vascular networks originate and branch from pre-existing vessels. It takes place mainly during early embryogenesis, while in adults blood vessels rarely form new brunches, except in tissue repair or disease conditions, including cancer progression. It involves the migration of endothelial cells at the lead of growing vessels, lumen formation and the maturation of newly formed blood vessels through the recruitment of mural cells and the consolidation of cell to cell adhesion [20]. The rapid development of new vascular networks is necessary to support the progression of cancer and therefore sustain neoplastic growth, while these events also facilitate the dissemination of metastases [21]. The most important angiogenic regulators are the vascular endothelial growth factor (VEGF) and its receptors, which are overexpressed in metastatic CRC [22]. Other mediators of angiogenesis include platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF) [23].

The VEGF family consists of five secreted glycoproteins (termed VEGF-A to -E) and the placenta growth factor (PlGF)-1 and -2, which bind, with different affinity and specificity, to three receptor tyrosine kinases (RTKs) on endothelial cells (termed VEGFR-1 to -3) [24,25]. VEGF promotes angiogenesis in several ways, which are mediated by intracellular signaling events initiated by the binding and dimerization of cognate receptors on endothelial cells. It has been shown that VEGF can induce vascular permeability through ERK1/2 (Extracel-

lular Signal-Regulated Kinase 1/2) and AKT signaling pathways, thus creating a pro-angiogenic environment [26]. Moreover, VEGF leads to upregulation of the anti-apoptotic BCL2 protein through the PI3K/AKT pathway and confers a survival signal to endothelial cells, while induces the secretion of key enzymes, such as metalloproteinases and other proteases, necessary for the migration and invasion of endothelial cells [27–29].

Upregulation and secretion of VEGF in the tumor microenvironment are mostly driven by hypoxia, which is the result of insufficient vascular supply inside the growing tumor. The hypoxia-inducible factors (HIFs) are upregulated during hypoxic conditions leading to the transactivation of angiogenesis-related genes (VEGF, PDGF-B) and cell proliferation (TGF-β) [30]. Besides hypoxia, HIF proteins are also upregulated through specific oncogenic signaling effectors, including ERK and PKA [31,32]. The production of VEGF can also be directly promoted by the activation of oncogenes, including *KRAS*, *HER2*, *EGFR* or members of the MAPK cascade, all of which can be found mutated in CRC [33–36]. Finally, VEGF expression can be mediated by several growth factors and cytokines, such as PDGF, IGF and prostaglandins [37–39].

Tumor vascular networks demonstrate high degrees of heterogeneity and atypical morphological features compared to normal vasculature. They are characterized by excessive permeability, poor perfusion and disorganized vascular pressure due to vascular immaturity and mechanical forces applied on the vessels by the growing tumor [40,41]. These events result in hypoxic areas that drive cancer cells to acquire a more aggressive phenotype [42]. In addition, numerous studies have documented the role of angiogenesis in colon cancer progression and metastasis, since it provides a conduit for cancer cell dissemination [43]. In this vein, not only the VEGFR-VEGF axis, but also other mechanisms, such as Notch signaling activation and E-selectin expression, are recruited [44,45]. Moreover, this heterogeneity has a direct impact on the efficacy of the available treatment options. Cells under hypoxic conditions are less sensitive to radiation, while insufficient blood supply of specific areas inside the tumor limits the delivery of chemotherapeutic agents and host immune cells triggered by immunotherapies to target cancer cells [46–48].

Targeting angiogenesis in colorectal cancer

Targeting angiogenesis is a major approach in cancer treatment. Although in metastatic CRC and most other cancer types, angiogenesis is not a determinant of cancer progression, anti-angiogenic treatment options show significant clinical activity [49]. Besides the reduction of tumor growth and inhibition of metastasis, they can also normalize vascular permeability and facilitate the delivery of chemotherapeutic agents, resulting in more effective cancer treatment [50].

The overall clinical benefit has been well established, even if it is slight. The two categories of medicines that target angiogenesis include monoclonal antibodies (mAbs) and tiny chemicals, such as tyrosine kinase inhibitors (TKIs) [51]. The mAbs act by either directly binding to VEGF-A or blocking the appropriate receptor's extracellular binding domain. Three mAbs are used in clinical practice: bevacizumab, aflibercept, and ramucirumab. Bevacizumab is a humanized IgG monoclonal antibody that binds to all isoforms of VEGF-A. Aflibercept is a soluble decoy receptor that binds to VEGF and stops it from activating its native receptors. Ramucirumab binds with a high affinity to the VEGFR-2 extracellular domain, preventing VEGF ligands from binding and thereby blocking receptor activation. TKIs work by binding to and inhibiting the kinase domains of a variety of receptors involved in the angiogenesis process [51,52].

Angiogenesis can be targeted for the management of mCRC in any line of treatment. Bevacizumab has been combined with chemotherapy in both first and second line of treatment, while aflibercept and ramucirumab have been approved for second-line treatment. In addition, regorafenib (TKI) is used as monotherapy in patients with chemo-resistant illness [53,54].

Bevacizumab is the most widely used anti-angiogenic inhibitor. Since monotherapy has a minor effect, it is frequently used with chemotherapy to improve efficacy as evaluated by the response rate (RR), progression-free survival (PFS), and overall survival (OS). It has been shown that when paired with chemotherapy, it outperforms the chemotherapy plus placebo [55–60]. Bevacizumab is used in conjunction with modern combination therapies. It appears to be more effective with the triplet FOLFOXIRI (folinic acid, 5-fluorouracil, oxaliplatin and irinotecan) than with FOLFIRI (folinic acid, 5-fluorouracil and irinotecan) alone [61]. After first-line treatment, bevacizumab is also effective when paired with chemotherapy [62–64]. Unfortunately, there are no clinically validated biomarkers for predicting bevacizumab benefit. In addition, bevacizumab can cause vascular adverse effects, the most dangerous of which

are gastrointestinal perforation, bleeding, and arterial thrombosis (<1% of patients). Furthermore, proteinuria, hypertension, and leukopenia are also common side effects [65,66].

In addition, ramucirumab is an approved medication for the second-line treatment of mCRC. In comparison to FOLFIRI alone, the combination of ramucirumab with FOLFIRI increased PFS and OS but not response rate [67]. On the other hand, in a phase II trial, it was discovered that adding ramucirumab to the FOLFOX (fluorouracil-leucovorin-oxaliplatin) regimen did not improve PFS [68]. Furthermore, aflibercept binds to VEGF-A more effectively than bevacizumab [69]. When combined with FOLFIRI, aflibercept improves survival in patients who had previously progressed on an oxaliplatin-containing therapy [70]. In the first-line scenario, however, the combination of aflibercept and FOLFOX did not produce any apparent improvement. As a result, aflibercept is used as a second-line CRC treatment [71].

Anti-angiogenic TKIs have also been evaluated in people with mCRC. Regorafenib is the only TKI that is used in the clinical practice of mCRC. CORRECT trial confirmed a survival benefit for regorafenib as monotherapy (median OS 6.4 months) compared to placebo group (5 months) [72]. A similar benefit over regorafenib (median OS 8.8 vs 6.3 months) was also confirmed in the CONCUR phase III clinical trial, in which only Asian patients were recruited [73]. Rash, fatigue, hand-foot skin response, anorexia and diarrhea are the most common adverse reactions in patients treated with regorafenib, and dose reductions are frequently required to manage regorafenib-related adverse events. A lower initial dose with a gradual dose increase has been proven in several trials to be an alternate, safe, and well-tolerated route to regorafenib administration, and this approach should be favored in daily practice [74,75].

RAS/RAF wild type and anti-EGFR therapies

The increased presence of the EGFR on cancerous tissue of the colon and rectum was detected about 35 years ago [76]. Since then, great progress has been made in the understanding of its involvement in disease pathogenesis, while two targeted biological agents have been approved and are widely employed in clinical practice.

Cetuximab and panitumumab represent the only approved anti-EGFR targeted therapies for metastatic colorectal cancer, with equivalent efficacy [77]. They are monoclonal antibodies that either bind extracellularly and downregulate EGFR and, subsequently, its tumor-promoting signaling or induce cancer cell death by mediating antibody-dependant cytotoxicity (ADCC) [78]. They also display a synergistic effect in combination with chemotherapy. In randomized controlled trials on the metastatic setting of colorectal cancer, cetuximab monotherapy increases overall and progression-free survival in chemotherapy pre-treated patients [79], while its addition to the pre-existing fluorouracil plus irinotecan combination can be used as first-line to reduce progression risk [80]. Similarly, adding panitumumab to fluorouracil/leucovorin plus oxaliplatin results in longer progression-free survival [81].

Previous and ongoing research on other anti-EGFR strategies has yielded mixed results. EGFR tyrosine kinase inhibitors have been hypothesized to inhibit EGFR-regulated pathways, as in the case of KRAS-wt Non-Small Cell Lung Cancer (NSCLC) [82]. Their success however was not repeated in early trials of gefitinib plus chemotherapy [83,84], while erlotinib has been proven more promising in increasing survival in KRAS-wt metastatic colorectal cancer [85], but these results were not consistent with those of other studies [86]. High toxicity was the common denominator among all studies [83–87].

EGFR itself is less important as a predictive marker of response and anti-EGFR therapies are indicated regardless of its degree of expression [88,89]. On the other hand, the absence of KRAS mutations, especially in exon 2, is a prerequisite for the administration of anti-EGFR targeted therapy, which is otherwise not only ineffective [90,91] but has been shown to expedite terminal outcomes [81]. This is attributed to bypassing EGFR signaling and activating the RAS/RAF/MAPK signaling pathway, enabled by the mutant variants [78]. Similarly, human epidermal growth factor receptor 2 (HER2) amplification is associated with shorter progression-free survival [92], possibly due to the EGFR-independent downstream activation of PI3K/AKT/mTOR and RAS/RAF/MAPK cascade or by heterodimerization with EGFR [93].

Interestingly, the location of colon cancer is of prognostic and predictive value. Based on data from the CRYSTAL [80] and FIRE-3 [94] randomized controlled trials, patients with left-sided RAS-wt metastatic colon cancer clearly benefit more from cetuximab plus chemotherapy in terms of response rates and survival than patients with right-sided tumors [95]. Right-sided tumors generally have a worse prognosis regardless of the interventions used [96] and display different histopathological and molecular characteristics compared to left-sided tumors [97],

including less robust EGFR signaling, that could explain the inefficiency of anti-EGFR strategies.

KRAS as a target

As we mentioned above, RAS is a protein family of 3 members KRAS (Kirsten rat sarcoma virus), NRAS (neuroblastoma RAS), and HRAS (HRas Proto-Oncogene, GTPase) that have GTPase function at the signal transduction of most growth factor receptors such as the EGFR [98]. *RAS* activating mutations and especially *KRAS* mutations are the most common genetic alterations in human carcinomas, accounting for almost one million deaths every year worldwide. It is found in about 40% of CRCs and has an anatomic specificity to the more aggressive right-sided tumors as compared to left-sided ones that are more likely to have *EFGR* mutations [99]. Mutations mostly in codons 12, 13, 61 in the *RAS* gene result in different *KRAS* mutant alleles with the most common ones for CRC being *G12D*, *G12V*, *G13D*, *G12A*, *G12S*, and *G12C*. The majority of the above mutations are caused by a single amino acid substitution.

Therapies targeting KRAS would be very effective for colorectal malignancies but unfortunately creating such an inhibitor is rather difficult and none has yet been approved. For now, the only drugs inhibiting KRAS directly are sotorasib (AMG510) and adagrasib (MRTX849). They bind to the P2 pocket of the switch I/II region of KRAS and lock it in its inactive form. Sotorasib is only approved for patients with advanced or metastatic NSCLC positive to the KRAS G12C mutation that have been previously treated with at least one other therapy [100]. Currently, multiple clinical trials in phases I and II examine the use of sotorasib in CRC as well. Specifically, the CodeBreak100 trial [101] ended up to the conclusion that by using sotorasib, malignancies can be controlled, and patients may benefit with up to 5.4 months of stable disease duration. The Krystal-1 trial [102] concluded that Adagrasib can also have therapeutic effects on patients with CRC, especially when combined with anti-EGFR treatments. Another compound that is being studied is BI-2852 that also binds at the same region of KRAS G12C mutation making it unable to bind with SOS1 and its effectors PI3K and RAF at different doses. Both MAPK and PI3K/AKT pathways were blocked producing antiproliferative effects in mutant cells. These studies confirmed that KRAS can be targeted directly and research in that direction should continue.

A recent study came up with a SOS1 (Son of Sevenless) inhibitor (BI-3406) [103] that blocks the binding of SOS1 to RAS when *KRAS* alleles *G12* (especially *G12D, G12V, and G12C*) and *G13D* as well are present. As a result, RAS cannot be activated leading to the blockage of cell proliferation. The combination of BI-3406 with a MEKI also known as MAPKI (Mitogen-activated protein kinase inhibitor) I- trametinib was also tested. The discovery of this compound and other analogs like BI 1701963 (phase I clinical trial NCT04111458) show a very promising therapeutic potential.

B Raf Kinase (BRAF) as a target

BRAF protein is a member of a serine-threonine kinase family. RAF kinases act mainly through phosphorylation and play an important role in many cellular processes, such as cell proliferation, differentiation and regulation of transcription. The proto-oncogene *BRAF*, which encodes the corresponding serine-threonine protein kinase, plays a critical role in the carcinogenesis not only of colorectal cancer, where it is mutated in 5% -8% of cases, but also in many other forms of the disease [104]. In particular, mutations in the gene can either be inherited or appear later in life and cause cancer [105]. Gene mutations show great diversity and many have been detected in large numbers (over 30) [106]. These can explain the development and progression of many malignancies. Typical examples are melanoma, NSCLC, colon cancer, papillary thyroid carcinoma, glioblastoma and astrocytoma [104,107,108].

Mutations may occur in different regions of the gene sequence. However, the most commonly identified mutations in colorectal cancer (and other malignancies) involve the replacement of thymine by adenine at nucleotide 1799, leading to the replacement of valine (V) by glutamate (E) at code 600 [108]. This mutation typically affects women, older people, smokers and more often right colon cancers [106,109]. Obviously, this mutation can play a crucial role in patients' prognosis and response to treatment. This is because it usually involves low-grade, advanced cancers that have already had lymph node metastases and perineural infiltration [109]. In addition, this mutation is associated in some studies with poor prognosis and may adversely affect patients with hepatic and pulmonary metastasectomy [107,110,111]. It is also negatively associated with the response to anti-EGFR agents [109]. Other mutations which have been found are G463E, G463V, G465A, G465E, G465V, G468A, D593V, F594L, etc. [112].

Before the era of BRAF targeting, intensive chemotherapy combined with anti-VEGF therapies was the

most appropriate approach for patients with BRAF-V600E [113]. However, during recent years, numerous of studies, which have evaluated different BRAF inhibitors as well as different combinations, have provided adequate evidence regarding the clinical significance of BRAF blockade in colorectal cancer. It is well documented that monotherapy with a BRAF-V600E inhibitor doesn't improve significantly response rates as well as that only simultaneously targeting at multiple steps provides clinically significant results. So, different combinations of BRAF inhibitors with anti-EGFR monoclonal antibodies and/or MEK inhibitors seem to have achieved the most promising results. Briefly, according to the BEACON trial, the combination of encorafenib (BRAF inhibitor) and cetuximab with or without binimetinib (a MEK inhibitor) in pre-treated patients with metastatic CRC improved clinical outcomes with a tolerable toxicity [114]. In addition, the use of vemurafenib (another BRAF inhibitor) together with cetuximab and irinotecan in patients with metastatic CRC has also showed positive results in terms of response to treatment and progression-free survival [115]. Furthermore, G. Middleton et al. have reported that the dabrafenib-trametinib-panitumumab combination produces a relatively satisfactory response in patients with BM1 gene expression profile, which represents 30% of all BRAF-V600E mutant CRC. These patients are characterized by increased potential for metastasis, activation of KRAS/AKT (AKT Serine/Threonine Kinase) signaling, stronger immune response and resistance to chemotherapy. On the other hand, in patients with BM2 disease (V600E mutation, deregulation of cell cycle control points, enrichment in metabolic processes), it was less beneficial [116]. Many other combinations have been studied or are under investigation, while other open questions are the best sequence strategy as well as the significance of the combination of target therapy with immunotherapy [113].

Microsatellite instability-high (MSI-H) tumors

The DNA replication mechanism is well conserved and maintained mainly due to the DNA polymerase's activity. However, the enzyme cannot always detect and repair its errors. In such a case, the MMR (mismatch repair) mechanism, among others, plays a crucial role [117]. Throughout the human genome, there are many short repetitive loci of DNA, called STRs (short tandem repeats) or microsatellites, which consist of one to six nucleotide repeats [118]. The term MSI refers to the presence of altered microsatellites' length (either longer or shorter) due to a defective MMR mechanism caused by mutations or epigenetic changes in one of its fundamental genes such as MSH2 (MutS Homolog 2), MSH6 (MutS Homolog 6), MLH1 (MutL Homolog 1), PMS1 (PMS1 protein homolog 1) and PMS2 (PMS2 protein homolog 2) [119]. In 1997, the American National Cancer Institute (NCI) suggested the classification of MSI status based on the number of detected mutated loci. More specifically, five significant loci used as biomarkers (BAT 25, BAT 26, D2S123, D5S346, and D17S250) are being examined and tumor status is classified as follows: MSI-H (Microsatellite Instability High) in the presence of two or more mutations detected, MSI-L (MSI low) when there is one and MSS (Microsatellite stable) when there is none [120].

MSI is considered as a hallmark of Lynch Syndrome (LS). Approximately 90% of LS cases are characterized by germline mutations of MMR genes, inherited in an autosomal dominant manner. LS predisposes to carcinogenesis at a younger age (before 50 years old) mainly of the colon, stomach and endometrium [121]. Screening of LS includes testing for MSI based on the Amsterdam Criteria [122] or the Bethesda Guidelines [123]. However, it is known that MSI does not take place early during tumorigenesis and only half of the colon adenomas examined will test positive [124], being indicative for LS since it is a rare finding in sporadic CRC. MSI is relatively frequent in sporadic CRC since 15% of the cases show deficient MMR (dMMR) mechanism. Hypermethylation of the MLH1 promoter constitutes the most common cause of dMMR sporadic CRC. This epigenetic alteration is often combined with BRAF V600E mutation but only in MSI-H sporadic CRC. Hence, when such a situation is identified, it contributes to the differential diagnosis between sporadic CRC and LS [125].

The MSI-H phenotype appears to have distinctive clinicopathological and histological features compared to MSI-L and MSS. These tumors mostly arise in the proximal colon (right-sided location) and are characterized by poor differentiation, high tumor infiltrating lymphocytes (TILs) counts and mucinous cell type [126]. An early-stage diagnosed MSI-H CRC has a favorable prognosis in comparison with MSS or MSI-L. Prominent lymphocytic infiltration of an MSI-H tumor suggests a high antitumor immune response leading to apoptosis, a result that probably explains the improved prognosis [127]. Nowadays, the MSI status of a CRC contributes to a more individualized selection of therapy. Ribic et al. concluded that MSI-H CRC do not benefit from fluorouracil-based adjuvant chemotherapy [128].

Immunotherapy in microsatellite instability-high (MSI-H) patients

The three primary treatment methods of metastatic colorectal cancer (mCRC), surgery, chemotherapy and radiotherapy remain the standard of care but their benefits have already reached a plateau. Therefore, it is urgent to develop a new effective therapeutic strategy to improve the survival outcome of cancer patients. At present, immunotherapy and targeted therapy are promising treatment strategies for CRC, since they have the potential to provide improved therapeutic efficacy with limited toxicity. Following the successful results of immunotherapy in other types of cancer, the interest in its use in CRC is highly increased and continuously growing [129].The way immunotherapy fights cancer is by stimulating the immune system against tumors. There are several categories of immunotherapy for many cancer types: adoptive cell therapy, cancer vaccines, oncolytic virus therapy, targeted antibodies, immunomodulators, etc. To date, immunomodulators have already been approved by the Food and Drug Administration (FDA) for the treatment of patients with dMMR/MSI-H mCRC and seem to be the most promising solution. One of the main representatives of immunomodulators are immune checkpoint inhibitors (ICIs) which regulate the interaction between T cells, antigen-presenting cells (APCs), and tumor cells to boost the release of suppressed immune responses. ICIs target co-inhibitory receptors, such as programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) expressed on T-cells and other immune-cell subpopulations, or their ligands, such as programmed cell death protein 1 ligand 1 (PD-L1) expressed on tumor cells and various immune cells [130]. The very high mutation rate in dMMR/MSI-H mCRC leads to the production and accumulation of hundreds of somatic mutations which results in a highly effective neoantigen presentation that attracts T-effector cells, such as CD8+ TILs, T helper 1 (Th1) CD4+ TILs and macrophages, as well as immunosuppressive cells such as myeloid-derived suppressor cells (MDSCs) and T-regulatory (Tregs) cells. In addition, these tumor cells exhibit upregulation of several immune checkpoint regulators such as PD-1, PD-L1, CTLA-4, Lymphocyte activation gene 3 (LAG3). That explains the high response rates and high sensitivity observed in dMMR/MSI-H mCRC patients treated with ICIs [131].

Two early phase II studies, KEYNOTE-016 and 164 evaluated single-agent pembrolizumab (anti-PD1) in previously treated dMMR/MSI-H mCRC. Patients in KEYNOTE-164 were divided into two cohorts, ≥2 (cohort A) or ≥ 1 (cohort B) prior lines of therapy (fluoropyrimidine, oxaliplatin, irinotecan, or anti-VEGF/EGFR). Pembrolizumab, at a flat dose of 200 mg every 3 weeks, was administered with a disease control rate (DCR) of 51% and 57% for cohorts A and B, respectively. The immune-related objective response rate (ORR) was 33% (N=124) [132]. On the other hand, in KEYNOTE-016 patients were divided into three separate cohorts: dMMR/MSI-H CRCs, pMMR/MSI-L CRCs, and dMMR/MSI-H non-CRCs, they were administered with a 10 mg/kg dose of pembrolizumab every 14 days. This study highlighted the different activity of pembrolizumab in CRC based on MMR status; the PFS at 20 weeks was 78% in dMMR/MSI-H CRC vs 11% in pMMR/MSI-H CRC and the ORR was 40% and 0%, respectively. An interesting point of this research was that the number of somatic mutations was significantly correlated with the chance of achieving response to therapy [133,134]. Pembrolizumab showed significant efficacy in the refractory setting following chemotherapy in patients with dMMR/MSI-H mCRC and was approved by the U.S. FDA for this indication, in 2017.

KEYNOTE-177 is a phase III, open-label trial, whose results led to the approval of pembrolizumab as monotherapy for the frontline treatment of patients with unresectable or metastatic, dMMR or MSI-H CRC. In this study, investigators compared the efficacy of first-line pembrolizumab monotherapy (N=153) vs standard of care chemotherapy ± bevacizumab or cetuximab (N=154) in 307 patients affected by dMMR/MSI-H mCRC. Pembrolizumab was superior to standard chemotherapy in terms of PFS (median 16.5 months vs 8.2 months, hazard ratio 0.60, 95% CI, 0.45-0.80, p = 0.0002) with a lower rate of treatment-related adverse events (AEs) (G3-5 AEs 22% versus 66%). Also, the ORR was 43,8% vs 33,1% for pembrolizumab and chemotherapy, respectively [135,136].

Immune checkpoints have been found to be overexpressed in dMMR/MSI CRCs compared to pMMR/MSS CRCs [137]. Then, combinations of monoclonal antibodies should be a solution to avoid primary resistance of dMMR/MSI-H mCRC to pembrolizumab. The FDA also approved nivolumab (anti-PD1) either alone or in combination with low dose ipilimumab (anti-CTLA4), for patients with dMMR/MSI-H mCRC, based on the results of the CheckMate 142 study. In this study, the researchers assessed the efficacy of nivolumab as first-line monotherapy (N=74) in comparison with the

combination of nivolumab with ipilimumab (N=119) and demonstrated very promising results, ORR 23% vs 55%, DCR 69% vs 80% and G3-4 AE rates 21% vs 32%, respectively [138,139]. Several ongoing clinical trials investigate the impact of the combination of immunotherapy and chemotherapy in dMMR/MSI-H mCRC patients, such as the COMMIT study which has 3 treatment arms, atezolizumab (anti-PD-L1) monotherapy vs FOLFOX + bevacizumab (anti-VEGF) vs atezolizumab + FOLFOX + bevacizumab [140]. Similarly, CheckMate 8HW is an ongoing study of nivolumab with or without ipilimumab or investigator's choice chemotherapy in dMMR/MSI-H mCRC patients [141]. Moreover, avelumab (anti-PD-L1) is investigated as an option in the second-line setting. In the SAMCO study avelumab is compared with standard of care in dMMR/MSI-H mCRC patients [142].

At present, many clinical trials investigate the efficacy of ICIs in combination with targeted therapies such as anti-VEGF drugs, anti-EGFR drugs, MAPK pathway inhibitors and multitarget kinase inhibitors. Moreover, the modulation of gut microbiota or fecal microbiota transplant seem to be promising options for boosting immunotherapy, in patients with dMMR/MSI-H mCRC with secondary resistance to ICIs (NCT03775850) [143]. Moreover, vaccination with frameshift peptides is one more option as a strategy to exaggerate primary or secondary resistance to ICIs in these patients (NCT04041310) [144–146].

Human epidermal growth factor receptor 2 (HER2) as a target

HER2 amplification occurs in 5% of mCRC patients [147]. The clinical significance of HER2 regarding its prognostic value in CRC needs further clarification. Early studies proposed a negative prognostic impact of HER2 overexpression, but more recent trials didn't confirmed the association between HER2 amplification and outcome [148,149].

According to the PETACC3 adjuvant chemotherapy trial and the subsequent DNA copy number & gene expression analysis, proximal carcinomas (ascending, hepatic flexure, transverse colon) were less likely to be HER2 or EGFR amplified compared to distal carcinomas (splenic flexure, descending colon, rectum) [97]. HER2 amplification in mCRC is enriched in KRAS, NRAS, BRAF and PIK3CA WT tumors and is a resistance marker for EGFR antibody therapy [150]. HER2 positive patients show more frequently lung metastases and higher tumor burden as well as HER2 positive tumors were more likely to be left sided [151]. Furthermore, HER2 status is also a molecular predictive biomarker for anti-HER2 targeted therapies (Trastuzumab/pertuzumab or Trastuzumab/lapatinib) [152].

The clinical significance of HER2 amplification regarding HER2-targeted therapies in patients with mCRC has been confirmed in many clinical trials (Figure 1). TRIUMPH was a phase II trial, in which circulating tumor

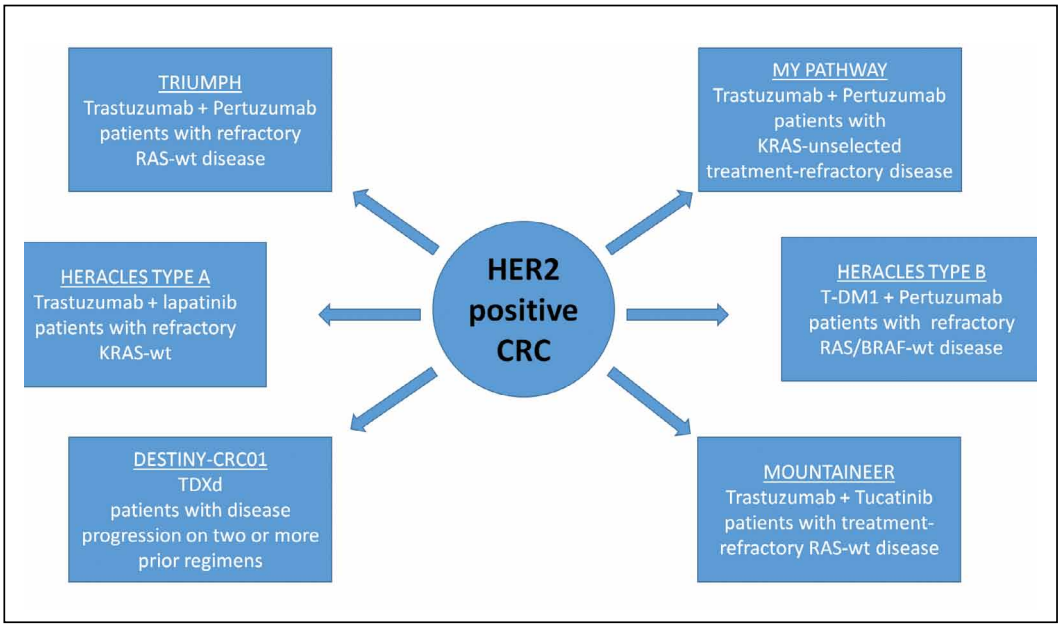

Figure 1. Clinical trials in which anti-HER2 treatments have been evaluated.

DNA (ctDNA) and simultaneously tissue HER2 testing were used [153]. The aim was to recognize patients for dual-HER2 blockade treatment with pertuzumab plus trastuzumab in patients with HER2 amplification and RAS wild-type. The outcomes of this investigation with pertuzumab / trastuzumab as a treatment in patients with chemorefractory RAS-WT disease (n=18) are ORR 35% (tissue positive), 33% (ctDNA-positive) and median progression free survival (mPFS) at 4 months. Findings from the TRIUMPH study confirmed the usefulness of ctDNA as a screening platform to select patients with HER2-amplified mCRC who will benefit from dual-HER2 blockade with trastuzumab and pertuzumab. The drawbacks of TRIUMPH are the small sample size and the use of a registry control arm [153].

In HERACLES-A trial, fluorescence in situ hybridization (FISH) and immunohistochemistry (IHC) were used. In this study, the above diagnostic algorithms were utilized to screen HER2-positive tumors for therapeutic targeting. More specifically, few patients had histologically confirmed KRAS-WT exon 2 (codons 12 and 13) and HER2-positive mCRC. Dual HER2 blockade with the combination of trastuzumab and oral lapatinib until disease progression or toxicity were investigated. The outcomes were ORR 28%, mPFS at 4.7 months in patients with increased gene copy number (GCN) > 9.5 and 3.7 months in patients with HER2 GCN <9.5 with median OS 10.0 months. The long-term (6.7 years) follow-up analysis of HERACLES-A provides strong evidence that the administration of trastuzumab and lapatinib combination in KRAS wild-type, chemorefractory HER2-positive mCRC patients provide survival as well as clinical benefits [154].

Another significant study was HERACLES-B study, which evaluated a targeted approach with a combination of pertuzumab and trastuzumab-emstasine (T-DM1) [155]. It was a single-arm, phase II trial in which patients with RAS/BRAF wild-type (n=31), HER2-amplified mCRC and refractory to standard treatments (chemorefractory) were enrolled. Diagnostic algorithms similar to that of HERACLES A were also used. At data cut-off, the ORR was 9.7%, the mPFS was 4.1 months and disease control rate 77.4%. Although, HERACLES-B trial did not reach its primary end point of ORR, low toxicity as well as high disease control rate support its therapeutic potential [155].

In addition, in MyPathway trial, which is a multiple basket, open-label, phase IIA study, pertuzumab in combination with trastuzumab was assessed in patients with HER2-amplified chemorefractory mCRC. ORR was 32%, mPFS 2.9 months and mOS 11.5 months. This trial confirmed the crucial role of HER2-targeted treatment with pertuzumab/trastuzumab and a chemotherapy-free regimen in patients with HER2-positive mCRC as well as the importance of molecular testing in colorectal cancer [156].

Two other studies also confirmed the role of anti-HER2 targeting in the management of mCRC. Firstly, MOUNTAINEER was a phase II trial in which tucatinib and trastuzumab was studied in patients with chemorefractory RAS-WT disease. Interim analysis showed a ORR 52.2%, mPFS 8.1 months and mOS 18.7 months [157]. In addition, DESTINY-CRC01 was a phase II trial in which the safety and antitumour activity of trastuzumab deruxtecan was investigated [158]. Patients with RAS and BRAF wild-type tumors and disease progression on two or more prior regimens (n=78) were enrolled into three cohorts according to the HER2 expression level (A, B, C classification with the assistance of IHC, ISH). The ORR in cohort A was 45.3%, (43.8% in patients who had previously received HER2-targeted therapy), DCR was 83%, mPFS 6.9 months while mOS wasn't reached. In addition, no responses were observed in cohorts B and C. This study showed that trastuzumab deruxtecan has a durable activity in HER2-positive mCRC refractory compared to standard treatments with a safe profile [158].

NTRK fusions in metastatic colorectal cancer

It is known that *NTRK1*, *NTRK2* and *NTRK3* genes encode the family of tropomyosin receptor kinases (TRK) TRKA, TRKB, TRKC, which are important for the neural system's normal development. When a nerve growth factor (NGF) is attached to a TRK protein, then the latter gets dimerized, phosphorylated and it activates the PI3K, RAS/MAPK/ERK and PLC-gamma signaling cascades. Alterations of the *NTRK* genes have been detected in many types of adult and children's solid tumors. Some examples of malignancies, in which *NTRK* gene rearrangements have been identified are thyroid [159], gliomas [160], lung [161] and colon [162] tumors.

Although the prevalence of *NTRK* gene alteration in colorectal cancer is below 1% - actually it is estimated that the incidence is between 0.23-0.97%- it is really important to identify these patients [163]. The detection of *NTRK* gene fusions can be identified by Next Generation Sequencing (NGS) with the use of RNA or DNA samples of the patient or by FISH. In 1986 the "*TPM3-TRK*" oncogene was found in a patient with colorectal cancer as a result of an intrachromosomal

rearrangement at 1q22-23. That leads to the fusion of the tropomyosin 3 gene (*TPM3*) with a sequence that encodes both transmembrane and intracellular parts of TRK receptor [164].

Larotrectinib was the first TRK inhibitor approved by FDA in November 2018 in the USA. Alexander Drilon and his team demonstrated through their clinical trial that Larotrectinib is efficient in adult and pediatric solid malignancies with *NTRK* fusions. In this clinical trial, 55 patients with *NTRK* positive tumors were treated with Larotrectinib, which is an orally administered small molecule that shows high affinity to the TRK receptor, without affecting other types of kinases. The ORR in this trial was 80% according to investigator's assessment and 75% according to central assessment. 13% of the patients had complete response (CR), while 62% showed partial response (PR). There were 4 patients with metastatic colorectal cancer and three of them responded to the treatment: two showed partial response and the other's disease remained stable. The median period of response was estimated to be 1.8 months. However, there were patients with primary resistance to Larotrectinib, as well as patients who had progressive disease and, as a result, new mutations that produced Larotrectinib resistance appeared. More specifically, these mutations were in the front position (NTRK1 G595R or NTRK3 G623R;), gatekeeper position (NTRK1 F589L;) and the xDFG position (NTRK1 G667S or NTRK3 G696A;) [165].

Entrectinib is also a 1st generation TRK inhibitor, orally administered, but unlike Larotrectinib that targets exclusively the TRK receptor, it is a pan-kinase inhibitor. That means that it also targets ALK and ROS1 proteins [166]. According to clinical trials, Entrectinib was proven to be well tolerated, while it caused clinically important response in patients with solid tumors characterized by *NTRK* positive fusion. Entrectinib showed efficacy in patients independently of whether they had central nervous system metastasis or not. Blinded Independent Central Review (BICR) showed 57.4% ORR and at the same time, 7.4% was CR. The median period of response was estimated to be 10.4 months [167].

Loxo-195 and Repotrectinb constitute the 2nd generation of TRK inhibitors and they were designed to target these mutations that are resistant to Larotrectinib and Entrectinib. Loxo-195 is selective to all three TRK kinases, their alterations and the acquired resistance mutations not only at preclinical level, but also in patients. A promising new therapy for patients with *NTRK* mutation starts with larotrectinib and is followed by LOXO-195 after the acquired resistance mutations appear. The target is to prolong the time period, during which the disease is under control [168].

CONCLUSIONS

During recent years, there is a plateau regarding the advances in the treatment of metastatic CRC compared to the advances in other solid tumors. Obviously, a better understanding of the underlying molecular mechanisms will lead to better characterization and exploitation of emerging new targets thus improving the management and treatment of CRC.

Conflict of interest disclosure: *None to declare*

Declaration of funding sources: *None to declare*

Author contributions: *FID & AK are responsible for conception, writing, data interpretation and review of the final draft of the article; All the authors contributed to the writing of the initial draft.*

REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71(3):209–49.
2. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer Statistics, 2021. CA Cancer J Clin. 2021;71(1):7–33.
3. Sawicki T, Ruszkowska M, Danielewicz A, Niedźwiedzka E, Arłukowicz T, Przybyłowicz KE. A Review of Colorectal Cancer in Terms of Epidemiology, Risk Factors, Development, Symptoms and Diagnosis. Cancers (Basel). 2021;13(9):2025.
4. Lee MKC, Loree JM. Current and emerging biomarkers in metastatic colorectal cancer. Curr Oncol. 2019;26(Suppl 1):S7–15.
5. Golshani G, Zhang Y. Advances in immunotherapy for colorectal cancer: a review. Therap Adv Gastroenterol. 2020;13:1756284820917527.
6. Mizukami T, Izawa N, Nakajima TE, Sunakawa Y. Targeting EGFR and RAS/RAF Signaling in the Treatment of Metastatic Colorectal Cancer: From Current Treatment Strategies to Future Perspectives. Drugs. 2019;79(6):633–45.
7. Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. Nat Rev Mol Cell Biol. 2001;2(2):127–37.
8. Miyamoto Y, Muguruma N, Fujimoto S, Okada Y, Kida Y, Nakamura F, et al. Epidermal growth factor receptor-targeted molecular imaging of colorectal tumors: Detection and treatment evaluation of tumors in animal models. Cancer Sci. 2019;110(6):1921–30.
9. El-Rayes BF, LoRusso PM. Targeting the epidermal growth factor receptor. Br J Cancer. 2004;91(3):418–24.

10. Khan K, Valeri N, Dearman C, Rao S, Watkins D, Starling N, et al. Targeting EGFR pathway in metastatic colorectal cancer- tumour heterogeniety and convergent evolution. Crit Rev Oncol Hematol. 2019;143:153–63.
11. Soung YH, Lee JW, Kim SY, Wang YP, Jo KH, Moon SW, et al. Somatic mutations of the ERBB4 kinase domain in human cancers. Int J Cancer. 2006;118(6):1426–9.
12. Han CW, Jeong MS, Jang SB. Structure, signaling and the drug discovery of the Ras oncogene protein. BMB Rep. 2017;50(7):355–60.
13. Durrant DE, Morrison DK. Targeting the Raf kinases in human cancer: the Raf dimer dilemma. Br J Cancer. 2018;118(1):3–8.
14. Cox AD, Fesik SW, Kimmelman AC, Luo J, Der CJ. Drugging the undruggable RAS: Mission possible? Nat Rev Drug Discov. 2014;13(11):828–51.
15. Wu P, Wee P, Jiang J, Chen X, Wang Z. Differential Regulation of Transcription Factors by Location-Specific EGF Receptor Signaling via a Spatio-Temporal Interplay of ERK Activation. Katz E, editor. PLoS One. 2012;7(9):e41354.
16. Pearson G, Robinson F, Beers Gibson T, Xu BE, Karandikar M, Berman K, et al. Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions. Endocr Rev. 2001;22(2):153–83.
17. McCubrey JA, Steelman LS, Chappell WH, Abrams SL, Wong EWT, Chang F, et al. Roles of the Raf/MEK/ERK pathway in cell growth, malignant transformation and drug resistance. Biochim Biophys Acta - Mol Cell Res. 2007;1773(8):1263–84.
18. Buday L, Warne PH, Downward J. Downregulation of the Ras activation pathway by MAP kinase phosphorylation of Sos. Oncogene. 1995;11(7):1327–31.
19. Eblen ST. Extracellular-Regulated Kinases: Signaling From Ras to ERK Substrates to Control Biological Outcomes. In 2018. p. 99–142.
20. Viallard C, Larrivée B. Tumor angiogenesis and vascular normalization: alternative therapeutic targets. Angiogenesis. 2017;20(4):409–26.
21. Hanahan D, Weinberg RA. Hallmarks of Cancer: The Next Generation. Cell. 2011;144(5):646–74.
22. Takahashi Y, Kitadai Y, Bucana CD, Cleary KR, Ellis LM. Expression of vascular endothelial growth factor and its receptor, KDR, correlates with vascularity, metastasis, and proliferation of human colon cancer. Cancer Res. 1995;55(18):3964–8.
23. Riechelmann R, Grothey A. Antiangiogenic therapy for refractory colorectal cancer: current options and future strategies. Ther Adv Med Oncol. 2017;9(2):106–26.
24. Hiratsuka S, Maru Y, Okada A, Seiki M, Noda T, Shibuya M. Involvement of Flt-1 tyrosine kinase (vascular endothelial growth factor receptor-1) in pathological angiogenesis. Cancer Res. 2001;61(3):1207–13.
25. Ferrara N, Gerber H-P, LeCouter J. The biology of VEGF and its receptors. Nat Med. 2003;9(6):669–76.
26. Lal BK, Varma S, Pappas PJ, Hobson RW, Durán WN. VEGF Increases Permeability of the Endothelial Cell Monolayer by Activation of PKB/akt, Endothelial Nitric-Oxide Synthase, and MAP Kinase Pathways. Microvasc Res. 2001;62(3):252–62.
27. Gerber H-P, McMurtrey A, Kowalski J, Yan M, Keyt BA, Dixit V, et al. Vascular Endothelial Growth Factor Regulates Endothelial Cell Survival through the Phosphatidylinositol 3′-Kinase/Akt Signal Transduction Pathway. J Biol Chem. 1998;273(46):30336–43.
28. Gerber H-P, Dixit V, Ferrara N. Vascular Endothelial Growth Factor Induces Expression of the Antiapoptotic Proteins Bcl-2 and A1 in Vascular Endothelial Cells. J Biol Chem. 1998;273(21):13313–6.
29. Wang H, Keiser JA. Vascular Endothelial Growth Factor Upregulates the Expression of Matrix Metalloproteinases in Vascular Smooth Muscle Cells. Circ Res. 1998;83(8):832–40.
30. Darby IA, Hewitson TD. Hypoxia in tissue repair and fibrosis. Cell Tissue Res. 2016;365(3):553–62.
31. Bullen JW, Tchernyshyov I, Holewinski RJ, DeVine L, Wu F, Venkatraman V, et al. Protein kinase A–dependent phosphorylation stimulates the transcriptional activity of hypoxia-inducible factor 1. Sci Signal. 2016;9(430).
32. Lim J-H, Lee E-S, You H-J, Lee JW, Park J-W, Chun Y-S. Ras-dependent induction of HIF-1α785 via the Raf/MEK/ERK pathway: a novel mechanism of Ras-mediated tumor promotion. Oncogene. 2004;23(58):9427–31.
33. Figueras A, Arbos MA, Quiles MT, Viñals F, Germà JR, Capellà G. The impact of KRAS mutations on VEGF-A production and tumour vascular network. BMC Cancer. 2013;13(1):125.
34. Laughner E, Taghavi P, Chiles K, Mahon PC, Semenza GL. HER2 (neu) Signaling Increases the Rate of Hypoxia-Inducible Factor 1α (HIF-1α) Synthesis: Novel Mechanism for HIF-1-Mediated Vascular Endothelial Growth Factor Expression. Mol Cell Biol. 2001;21(12):3995–4004.
35. Petit AM, Rak J, Hung MC, Rockwell P, Goldstein N, Fendly B, et al. Neutralizing antibodies against epidermal growth factor and ErbB-2/neu receptor tyrosine kinases down-regulate vascular endothelial growth factor production by tumor cells in vitro and in vivo: angiogenic implications for signal transduction therapy of so. Am J Pathol. 1997;151(6):1523–30.
36. Hoshi S, Nomoto K, Kuromitsu J, Tomari S, Nagata M. High Glucose Induced VEGF Expression via PKC and ERK in Glomerular Podocytes. Biochem Biophys Res Commun. 2002;290(1):177–84.
37. Cao R, Bråkenhielm E, Li X, Pietras K, Widenfalk J, Östman A, et al. Angiogenesis stimulated by PDGF-CC, a novel member in the PDGF family, involves activation of PDGFR-aa and -ap receptors. FASEB J. 2002;16(12):1575–83.
38. Miele C, Rochford JJ, Filippa N, Giorgetti-Peraldi S, Van Obberghen E. Insulin and Insulin-like Growth Factor-I Induce Vascular Endothelial Growth Factor mRNA Expression via Different Signaling Pathways. J Biol Chem. 2000;275(28):21695–702.
39. Hatazawa R, Tanigami M, Izumi N, Kamei K, Tanaka A, Takeuchi K. Prostaglandin E2 stimulates VEGF expression in primary rat gastric fibroblasts through EP4 receptors. Inflammopharmacology. 2007;15(5):214–7.
40. Morikawa S, Baluk P, Kaidoh T, Haskell A, Jain RK, McDonald DM. Abnormalities in Pericytes on Blood Vessels and En-

dothelial Sprouts in Tumors. Am J Pathol. 2002;160(3):985–1000.
41. Jain RK, Martin JD, Stylianopoulos T. The Role of Mechanical Forces in Tumor Growth and Therapy. Annu Rev Biomed Eng. 2014;16(1):321–46.
42. Reymond N, D'Água BB, Ridley AJ. Crossing the endothelial barrier during metastasis. Nat Rev Cancer. 2013;13(12):858–70.
43. Li J, Chen D, Shen M. Tumor Microenvironment Shapes Colorectal Cancer Progression, Metastasis, and Treatment Responses. Front Med. 2022;9.
44. Lu J, Ye X, Fan F, Xia L, Bhattacharya R, Bellister S, et al. Endothelial Cells Promote the Colorectal Cancer Stem Cell Phenotype through a Soluble Form of Jagged-1. Cancer Cell. 2013;23(2):171–85.
45. Nübel T, Dippold W, Kleinert H, Kaina B, Fritz G. Lovastatin inhibits Rho-regulated expression of E-selectin by TNF-α and attenuates tumor cell adhesion. FASEB J. 2004;18(1):140–2.
46. Busk M, Horsman MR. Relevance of hypoxia in radiation oncology: pathophysiology, tumor biology and implications for treatment. Q J Nucl Med Mol Imaging. 2013;57(3):219–34.
47. Heldin C-H, Rubin K, Pietras K, Östman A. High interstitial fluid pressure — an obstacle in cancer therapy. Nat Rev Cancer. 2004;4(10):806–13.
48. Huang Y, Goel S, Duda DG, Fukumura D, Jain RK. Vascular Normalization as an Emerging Strategy to Enhance Cancer Immunotherapy. Cancer Res. 2013;73(10):2943–8.
49. Sun W. Angiogenesis in metastatic colorectal cancer and the benefits of targeted therapy. J Hematol Oncol. 2012;5(1):63.
50. Bagnasco L, Piras D, Parodi S, Bauer I, Zoppoli G, Patrone F, et al. Role of Angiogenesis Inhibitors in Colorectal Cancer: Sensitive and Insensitive Tumors. Curr Cancer Drug Targets. 2012;12(4):303–15.
51. Hansen TF, Qvortrup C, Pfeiffer P. Angiogenesis Inhibitors for Colorectal Cancer. A Review of the Clinical Data. Cancers (Basel). 2021;13(5).
52. Xie Y-H, Chen Y-X, Fang J-Y. Comprehensive review of targeted therapy for colorectal cancer. Signal Transduct Target Ther. 2020;5(1):22.
53. Van Cutsem E, Cervantes A, Adam R, Sobrero A, Van Krieken JH, Aderka D, et al. ESMO consensus guidelines for the management of patients with metastatic colorectal cancer. Ann Oncol Off J Eur Soc Med Oncol. 2016;27(8):1386–422.
54. Pfeiffer P, Köhne C-H, Qvortrup C. The changing face of treatment for metastatic colorectal cancer. Expert Rev Anticancer Ther. 2019;19(1):61–70.
55. Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med. 2004;350(23):2335–42.
56. Kabbinavar FF, Schulz J, McCleod M, Patel T, Hamm JT, Hecht JR, et al. Addition of bevacizumab to bolus fluorouracil and leucovorin in first-line metastatic colorectal cancer: results of a randomized phase II trial. J Clin Oncol. 2005;23(16):3697–705.
57. Kabbinavar F, Hurwitz HI, Fehrenbacher L, Meropol NJ, Novotny WF, Lieberman G, et al. Phase II, randomized trial comparing bevacizumab plus fluorouracil (FU)/leucovorin (LV) with FU/LV alone in patients with metastatic colorectal cancer. J Clin Oncol. 2003;21(1):60–5.
58. Guan Z-Z, Xu J-M, Luo R-C, Feng F-Y, Wang L-W, Shen L, et al. Efficacy and safety of bevacizumab plus chemotherapy in Chinese patients with metastatic colorectal cancer: a randomized phase III ARTIST trial. Chin J Cancer. 2011;30(10):682–9.
59. Stathopoulos GP, Batziou C, Trafalis D, Koutantos J, Batzios S, Stathopoulos J, et al. Treatment of Colorectal Cancer with and without Bevacizumab: A Phase III Study. Oncology. 2010;78(5–6):376–81.
60. Cunningham D, Lang I, Marcuello E, Lorusso V, Ocvirk J, Shin DB, et al. Bevacizumab plus capecitabine versus capecitabine alone in elderly patients with previously untreated metastatic colorectal cancer (AVEX): an open-label, randomised phase 3 trial. Lancet Oncol. 2013;14(11):1077–85.
61. Cremolini C, Loupakis F, Antoniotti C, Lupi C, Sensi E, Lonardi S, et al. FOLFOXIRI plus bevacizumab versus FOLFIRI plus bevacizumab as first-line treatment of patients with metastatic colorectal cancer: updated overall survival and molecular subgroup analyses of the open-label, phase 3 TRIBE study. Lancet Oncol. 2015;16(13):1306–15.
62. Giantonio BJ, Catalano PJ, Meropol NJ, O'Dwyer PJ, Mitchell EP, Alberts SR, et al. Bevacizumab in combination with oxaliplatin, fluorouracil, and leucovorin (FOLFOX4) for previously treated metastatic colorectal cancer: results from the Eastern Cooperative Oncology Group Study E3200. J Clin Oncol. 2007;25(12):1539–44.
63. Masi G, Salvatore L, Boni L, Loupakis F, Cremolini C, Fornaro L, et al. Continuation or reintroduction of bevacizumab beyond progression to first-line therapy in metastatic colorectal cancer: final results of the randomized BEBYP trial. Ann Oncol Off J Eur Soc Med Oncol. 2015;26(4):724–30.
64. Pfeiffer P, Yilmaz M, Möller S, Zitnjak D, Krogh M, Petersen LN, et al. TAS-102 with or without bevacizumab in patients with chemorefractory metastatic colorectal cancer: an investigator-initiated, open-label, randomised, phase 2 trial. Lancet Oncol. 2020;21(3):412–20.
65. Hurwitz HI, Tebbutt NC, Kabbinavar F, Giantonio BJ, Guan Z-Z, Mitchell L, et al. Efficacy and safety of bevacizumab in metastatic colorectal cancer: pooled analysis from seven randomized controlled trials. Oncologist. 2013;18(9):1004–12.
66. Zhu X, Wu S, Dahut WL, Parikh CR. Risks of Proteinuria and Hypertension With Bevacizumab, an Antibody Against Vascular Endothelial Growth Factor: Systematic Review and Meta-Analysis. Am J Kidney Dis. 2007;49(2):186–93.
67. Tabernero J, Yoshino T, Cohn AL, Obermannova R, Bodoky G, Garcia-Carbonero R, et al. Ramucirumab versus placebo in combination with second-line FOLFIRI in patients with metastatic colorectal carcinoma that progressed during or after first-line therapy with bevacizumab, oxaliplatin, and a fluoropyrimidine (RAISE): a randomised, double-blin. Lancet Oncol. 2015;16(5):499–508.

68. Moore M, Gill S, Asmis T, Berry S, Burkes R, Zbuk K, et al. Randomized phase II study of modified FOLFOX-6 in combination with ramucirumab or icrucumab as second-line therapy in patients with metastatic colorectal cancer after disease progression on first-line irinotecan-based therapy. Ann Oncol Off J Eur Soc Med Oncol. 2016;27(12):2216–24.
69. Tang PA, Cohen SJ, Kollmannsberger C, Bjarnason G, Virik K, MacKenzie MJ, et al. Phase II clinical and pharmacokinetic study of aflibercept in patients with previously treated metastatic colorectal cancer. Clin Cancer Res. 2012;18(21):6023–31.
70. Van Cutsem E, Tabernero J, Lakomy R, Prenen H, Prausová J, Macarulla T, et al. Addition of aflibercept to fluorouracil, leucovorin, and irinotecan improves survival in a phase III randomized trial in patients with metastatic colorectal cancer previously treated with an oxaliplatin-based regimen. J Clin Oncol. 2012;30(28):3499–506.
71. Folprecht G, Pericay C, Saunders MP, Thomas A, Lopez Lopez R, Roh JK, et al. Oxaliplatin and 5-FU/folinic acid (modified FOLFOX6) with or without aflibercept in first-line treatment of patients with metastatic colorectal cancer: the AFFIRM study. Ann Oncol Off J Eur Soc Med Oncol. 2016;27(7):1273–9.
72. Grothey A, Van Cutsem E, Sobrero A, Siena S, Falcone A, Ychou M, et al. Regorafenib monotherapy for previously treated metastatic colorectal cancer (CORRECT): an international, multicentre, randomised, placebo-controlled, phase 3 trial. Lancet (London, England). 2013;381(9863):303–12.
73. Li J, Qin S, Xu R, Yau TCC, Ma B, Pan H, et al. Regorafenib plus best supportive care versus placebo plus best supportive care in Asian patients with previously treated metastatic colorectal cancer (CONCUR): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol. 2015;16(6):619–29.
74. Bekaii-Saab TS, Ou F-S, Ahn DH, Boland PM, Ciombor KK, Heying EN, et al. Regorafenib dose-optimisation in patients with refractory metastatic colorectal cancer (ReDOS): a randomised, multicentre, open-label, phase 2 study. Lancet Oncol. 2019;20(8):1070–82.
75. Grothey A, Blay J-Y, Pavlakis N, Yoshino T, Bruix J. Evolving role of regorafenib for the treatment of advanced cancers. Cancer Treat Rev. 2020;86:101993.
76. Bradley SJ, Garfinkle G, Walker E, Salem R, Chen LB, Steele G. Increased expression of the epidermal growth factor receptor on human colon carcinoma cells. Arch Surg. 1986;121(11):1242–7.
77. Price TJ, Peeters M, Kim TW, Li J, Cascinu S, Ruff P, et al. Panitumumab versus cetuximab in patients with chemotherapy-refractory wild-type KRAS exon 2 metastatic colorectal cancer (ASPECCT): a randomised, multicentre, open-label, non-inferiority phase 3 study. Lancet Oncol. 2014;15(6):569–79.
78. García-Foncillas J, Sunakawa Y, Aderka D, Wainberg Z, Ronga P, Witzler P, et al. Distinguishing Features of Cetuximab and Panitumumab in Colorectal Cancer and Other Solid Tumors. Front Oncol. 2019;9:849.
79. Jonker DJ, O'Callaghan CJ, Karapetis CS, Zalcberg JR, Tu D, Au H-J, et al. Cetuximab for the Treatment of Colorectal Cancer. N Engl J Med. 2007;357(20):2040–8.
80. Van Cutsem E, Köhne C-H, Hitre E, Zaluski J, Chang Chien C-R, Makhson A, et al. Cetuximab and Chemotherapy as Initial Treatment for Metastatic Colorectal Cancer. N Engl J Med. 2009;360(14):1408–17.
81. Douillard J-Y, Siena S, Cassidy J, Tabernero J, Burkes R, Barugel M, et al. Randomized, phase III trial of panitumumab with infusional fluorouracil, leucovorin, and oxaliplatin (FOLFOX4) versus FOLFOX4 alone as first-line treatment in patients with previously untreated metastatic colorectal cancer: the PRIME study. J Clin Oncol. 2010;28(31):4697–705.
82. Sellmann L, Fenchel K, Dempke WCM. Improved overall survival following tyrosine kinase inhibitor treatment in advanced or metastatic non-small-cell lung cancer-the Holy Grail in cancer treatment? Transl lung cancer Res. 2015;4(3):223–7.
83. Santoro A, Comandone A, Rimassa L, Granetti C, Lorusso V, Oliva C, et al. A phase II randomized multicenter trial of gefitinib plus FOLFIRI and FOLFIRI alone in patients with metastatic colorectal cancer. Ann Oncol Off J Eur Soc Med Oncol. 2008;19(11):1888–93.
84. Kindler HL, Friberg G, Skoog L, Wade-Oliver K, Vokes EE. Phase I/II trial of gefitinib and oxaliplatin in patients with advanced colorectal cancer. Am J Clin Oncol. 2005;28(4):340–4.
85. Vincent MD, Breadner D, Soulieres D, Kerr IG, Sanatani M, Kocha W, et al. Phase II trial of capecitabine plus erlotinib versus capecitabine alone in patients with advanced colorectal cancer. Future Oncol. 2017;13(9):777–86.
86. Hagman H, Frödin J-E, Berglund Å, Sundberg J, Vestermark LW, Albertsson M, et al. A randomized study of KRAS-guided maintenance therapy with bevacizumab, erlotinib or metronomic capecitabine after first-line induction treatment of metastatic colorectal cancer: the Nordic ACT2 trial. Ann Oncol Off J Eur Soc Med Oncol. 2016;27(1):140–7.
87. Kearney MR, Chen EY, Vaccaro GM, Strother J, Burt A, Todd K, et al. A Phase II Study Alternating Erlotinib With Second-line mFOLFOX6 or FOLFIRI for Metastatic Colorectal Cancer. Anticancer Res. 2019;39(1):245–52.
88. Cunningham D, Humblet Y, Siena S, Khayat D, Bleiberg H, Santoro A, et al. Cetuximab Monotherapy and Cetuximab plus Irinotecan in Irinotecan-Refractory Metastatic Colorectal Cancer. N Engl J Med. 2004;351(4):337–45.
89. Saltz LB, Meropol NJ, Loehrer PJ, Needle MN, Kopit J, Mayer RJ. Phase II trial of cetuximab in patients with refractory colorectal cancer that expresses the epidermal growth factor receptor. J Clin Oncol. 2004;22(7):1201–8.
90. Amado RG, Wolf M, Peeters M, Van Cutsem E, Siena S, Freeman DJ, et al. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. J Clin Oncol. 2008;26(10):1626–34.
91. Karapetis CS, Khambata-Ford S, Jonker DJ, O'Callaghan CJ, Tu D, Tebbutt NC, et al. K-ras Mutations and Benefit from Cetuximab in Advanced Colorectal Cancer. N Engl J Med. 2008;359(17):1757–65.

92. Raghav K, Loree JM, Morris JS, Overman MJ, Yu R, Meric-Bernstam F, et al. Validation of HER2 Amplification as a Predictive Biomarker for Anti–Epidermal Growth Factor Receptor Antibody Therapy in Metastatic Colorectal Cancer. JCO Precis Oncol. 2019;(3):1–13.
93. La Thangue NB, Kerr DJ. Predictive biomarkers: a paradigm shift towards personalized cancer medicine. Nat Rev Clin Oncol. 2011;8(10):587–96.
94. Heinemann V, von Weikersthal LF, Decker T, Kiani A, Vehling-Kaiser U, Al-Batran S-E, et al. FOLFIRI plus cetuximab versus FOLFIRI plus bevacizumab as first-line treatment for patients with metastatic colorectal cancer (FIRE-3): a randomised, open-label, phase 3 trial. Lancet Oncol. 2014;15(10):1065–75.
95. Tejpar S, Stintzing S, Ciardiello F, Tabernero J, Van Cutsem E, Beier F, et al. Prognostic and Predictive Relevance of Primary Tumor Location in Patients With RAS Wild-Type Metastatic Colorectal Cancer: Retrospective Analyses of the CRYSTAL and FIRE-3 Trials. JAMA Oncol. 2017;3(2):194–201.
96. Modest DP, Schulz C, von Weikersthal LF, Quietzsch D, von Einem JC, Schalhorn A, et al. Outcome of patients with metastatic colorectal cancer depends on the primary tumor site (midgut vs. hindgut): analysis of the FIRE1-trial (FuFIRI or mIROX as first-line treatment). Anticancer Drugs. 2014;25(2):212–8.
97. Missiaglia E, Jacobs B, D'Ario G, Di Narzo AF, Soneson C, Budinska E, et al. Distal and proximal colon cancers differ in terms of molecular, pathological, and clinical features. Ann Oncol Off J Eur Soc Med Oncol. 2014;25(10):1995–2001.
98. Timar J, Kashofer K. Molecular epidemiology and diagnostics of KRAS mutations in human cancer. Cancer Metastasis Rev. 2020;39(4):1029–38.
99. Imperial R, Ahmed Z, Toor OM, Erdoğan C, Khaliq A, Case P, et al. Comparative proteogenomic analysis of right-sided colon cancer, left-sided colon cancer and rectal cancer reveals distinct mutational profiles. Mol Cancer. 2018;17(1):177.
100. Shahi Thakuri P, Lamichhane A, Singh S, Gupta M, Luker GD, Tavana H. Modeling Adaptive Resistance of KRAS Mutant Colorectal Cancer to MAPK Pathway Inhibitors with a Three-Dimensional Tumor Model. ACS Pharmacol Transl Sci. 2020;3(6):1176–87.
101. Kessler D, Gmachl M, Mantoulidis A, Martin LJ, Zoephel A, Mayer M, et al. Drugging an undruggable pocket on KRAS. Proc Natl Acad Sci U S A. 2019;116(32):15823–9.
102. Adagrasib Data Create Buzz at ESMO. Cancer Discov. 2021;11(11):2664.
103. Hofmann MH, Gmachl M, Ramharter J, Savarese F, Gerlach D, Marszalek JR, et al. BI-3406, a Potent and Selective SOS1-KRAS Interaction Inhibitor, Is Effective in KRAS-Driven Cancers through Combined MEK Inhibition. Cancer Discov. 2021;11(1):142–57.
104. Halle BR, Johnson DB. Defining and Targeting BRAF Mutations in Solid Tumors. Curr Treat Options Oncol. 2021;22(4):30.
105. Mármol I, Sánchez-de-Diego C, Pradilla Dieste A, Cerrada E, Rodriguez Yoldi M. Colorectal Carcinoma: A General Overview and Future Perspectives in Colorectal Cancer. Int J Mol Sci. 2017;18(1):197.
106. Barras D, Missiaglia E, Wirapati P, Sieber OM, Jorissen RN, Love C, et al. BRAF V600E Mutant Colorectal Cancer Subtypes Based on Gene Expression. Clin Cancer Res. 2017;23(1):104–15.
107. Guan W-L, Qiu M-Z, He C-Y, Yang L-Q, Jin Y, Wang Z-Q, et al. Clinicopathologic Features and Prognosis of BRAF Mutated Colorectal Cancer Patients. Front Oncol. 2020;10.
108. Fanelli GN, Dal Pozzo CA, Depetris I, Schirripa M, Brignola S, Biason P, et al. The heterogeneous clinical and pathological landscapes of metastatic Braf-mutated colorectal cancer. Cancer Cell Int. 2020;20(1):30.
109. Lièvre A, de la Fouchardière C, Samalin E, Benoist S, Phelip J-M, André T, et al. Cancers colorectaux avec mutation V600E de BRAF : où en sommes-nous ? Bull Cancer. 2020;107(9):881–95.
110. Ampollini L, Gnetti L, Goldoni M, Viani L, Faedda E, Campanini N, et al. Pulmonary metastasectomy for colorectal cancer: analysis of prognostic factors affecting survival. J Thorac Dis. 2017;9(S12):S1282–90.
111. Teng H-W, Huang Y-C, Lin J-K, Chen W-S, Lin T-C, Jiang J-K, et al. BRAF mutation is a prognostic biomarker for colorectal liver metastasectomy. J Surg Oncol. 2012;106(2):123–9.
112. Luu L-J, J. Price T. BRAF Mutation and Its Importance in Colorectal Cancer. In: Advances in the Molecular Understanding of Colorectal Cancer. IntechOpen; 2019.
113. Ros J, Baraibar I, Sardo E, Mulet N, Salvà F, Argilés G, et al. BRAF, MEK and EGFR inhibition as treatment strategies in BRAF V600E metastatic colorectal cancer. Ther Adv Med Oncol. 2021;13:175883592199297.
114. Van Cutsem E, Huijberts S, Grothey A, Yaeger R, Cuyle P-J, Elez E, et al. Binimetinib, Encorafenib, and Cetuximab Triplet Therapy for Patients With BRAF V600E–Mutant Metastatic Colorectal Cancer: Safety Lead-In Results From the Phase III BEACON Colorectal Cancer Study. J Clin Oncol. 2019;37(17):1460–9.
115. Kopetz S, Guthrie KA, Morris VK, Lenz H-J, Magliocco AM, Maru D, et al. Randomized Trial of Irinotecan and Cetuximab With or Without Vemurafenib in BRAF-Mutant Metastatic Colorectal Cancer (SWOG S1406). J Clin Oncol. 2021;39(4):285–94.
116. Middleton G, Yang Y, Campbell CD, André T, Atreya CE, Schellens JHM, et al. BRAF -Mutant Transcriptional Subtypes Predict Outcome of Combined BRAF, MEK, and EGFR Blockade with Dabrafenib, Trametinib, and Panitumumab in Patients with Colorectal Cancer. Clin Cancer Res. 2020;26(11):2466–76.
117. Liu D, Keijzers G, Rasmussen LJ. DNA mismatch repair and its many roles in eukaryotic cells. Mutat Res Mutat Res. 2017;773:174–87.
118. Garrido-Ramos M. Satellite DNA: An Evolving Topic. Genes (Basel). 2017;8(9):230.
119. Lin A, Zhang J, Luo P. Crosstalk Between the MSI Status and Tumor Microenvironment in Colorectal Cancer. Front

Immunol. 2020;11.
120. Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, et al. A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res. 1998;58(22):5248–57.
121. Geiersbach KB, Samowitz WS. Microsatellite Instability and Colorectal Cancer. Arch Pathol Lab Med. 2011;135(10):1269–77.
122. Vasen HFA, Mecklin J-P, Meera Khan P, Lynch HT. The International Collaborative Group on Hereditary Non-Polyposis Colorectal Cancer (ICG-HNPCC). Dis Colon Rectum. 1991;34(5):424–5.
123. Umar A, Boland CR, Terdiman JP, Syngal S, Chapelle A d. l., Ruschoff J, et al. Revised Bethesda Guidelines for Hereditary Nonpolyposis Colorectal Cancer (Lynch Syndrome) and Microsatellite Instability. JNCI J Natl Cancer Inst. 2004;96(4):261–8.
124. Stoffel EM, Syngal S. Adenomas in Young Patients: What is the Optimal Evaluation? Am J Gastroenterol. 2005;100(5):1150–3.
125. Yamamoto H, Imai K. Microsatellite instability: an update. Arch Toxicol. 2015;89(6):899–921.
126. Harada S, Morlote D. Molecular Pathology of Colorectal Cancer. Adv Anat Pathol. 2020;27(1):20–6.
127. Michael-Robinson JM. Tumour infiltrating lymphocytes and apoptosis are independent features in colorectal cancer stratified according to microsatellite instability status. Gut. 2001;48(3):360–6.
128. Ribic CM, Sargent DJ, Moore MJ, Thibodeau SN, French AJ, Goldberg RM, et al. Tumor Microsatellite-Instability Status as a Predictor of Benefit from Fluorouracil-Based Adjuvant Chemotherapy for Colon Cancer. N Engl J Med. 2003;349(3):247–57.
129. Robert C. A decade of immune-checkpoint inhibitors in cancer therapy. Nat Commun. 2020;11(1):3801.
130. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012;12(4):252–64.
131. Llosa NJ, Cruise M, Tam A, Wicks EC, Hechenbleikner EM, Taube JM, et al. The vigorous immune microenvironment of microsatellite instable colon cancer is balanced by multiple counter-inhibitory checkpoints. Cancer Discov. 2015;5(1):43–51.
132. Le DT, Kim TW, Van Cutsem E, Geva R, Jäger D, Hara H, et al. Phase II Open-Label Study of Pembrolizumab in Treatment-Refractory, Microsatellite Instability-High/Mismatch Repair-Deficient Metastatic Colorectal Cancer: KEYNOTE-164. J Clin Oncol. 2020;38(1):11–9.
133. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, et al. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. N Engl J Med. 2015;372(26):2509–20.
134. Le DT, Durham JN, Smith KN, Wang H, Bartlett BR, Aulakh LK, et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. Science. 2017;357(6349):409–13.
135. Andre T, Amonkar M, Norquist JM, Shiu K-K, Kim TW, Jensen BV, et al. Health-related quality of life in patients with microsatellite instability-high or mismatch repair deficient metastatic colorectal cancer treated with first-line pembrolizumab versus chemotherapy (KEYNOTE-177): an open-label, randomised, phase 3 trial. Lancet Oncol. 2021;22(5):665–77.
136. André T, Shiu K-K, Kim TW, Jensen BV, Jensen LH, Punt C, et al. Pembrolizumab in Microsatellite-Instability-High Advanced Colorectal Cancer. N Engl J Med. 2020;383(23):2207–18.
137. Zaravinos A, Roufas C, Nagara M, de Lucas Moreno B, Oblovatskaya M, Efstathiades C, et al. Cytolytic activity correlates with the mutational burden and deregulated expression of immune checkpoints in colorectal cancer. J Exp Clin Cancer Res. 2019;38(1):364.
138. Overman MJ, McDermott R, Leach JL, Lonardi S, Lenz H-J, Morse MA, et al. Nivolumab in patients with metastatic DNA mismatch repair-deficient or microsatellite instability-high colorectal cancer (CheckMate 142): an open-label, multicentre, phase 2 study. Lancet Oncol. 2017;18(9):1182–91.
139. Overman MJ, Lonardi S, Wong KYM, Lenz H-J, Gelsomino F, Aglietta M, et al. Durable Clinical Benefit With Nivolumab Plus Ipilimumab in DNA Mismatch Repair–Deficient/Microsatellite Instability–High Metastatic Colorectal Cancer. J Clin Oncol. 2018;36(8):773–9.
140. Lee JJ, Yothers G, Jacobs SA, Sanoff HK, Cohen DJ, Guthrie KA, et al. Colorectal Cancer Metastatic dMMR Immuno-Therapy (COMMIT) study (NRG- GI004/SWOG-S1610): A randomized phase III study of mFOLFOX6/bevacizumab combination chemotherapy with or without atezolizumab or atezolizumab monotherapy in the first-line treatment of. J Clin Oncol. 2018;36(15_suppl):TPS3615–TPS3615.
141. Abdullaev S, André T, Lei M, Lenz H-J, Novotny J, Paulson AS, et al. A phase III study of nivolumab (NIVO), NIVO + ipilimumab (IPI), or chemotherapy (CT) for microsatellite instability-high (MSI-H)/mismatch repair-deficient (dMMR) metastatic colorectal cancer (mCRC): Checkmate 8HW. J Clin Oncol. 2020;38(4_suppl):TPS266–TPS266.
142. Taïeb J, André T, El Hajbi F, Barbier E, Toullec C, Kim S, et al. Avelumab versus standard second line treatment chemotherapy in metastatic colorectal cancer patients with microsatellite instability: The SAMCO-PRODIGE 54 randomised phase II trial. Dig Liver Dis. 2021;53(3):318–23.
143. Gardner HA, Kashyap S, Ponichtera H, Sandy P, Parameswaran P, Carlson M, et al. Monoclonal microbial EDP1503 to induce antitumor responses via gut-mediated activation of both innate and adaptive immunity. J Clin Oncol. 2019;37(15_suppl):e14241–e14241.
144. Roudko V, Bozkus CC, Orfanelli T, McClain CB, Carr C, O'Donnell T, et al. Shared Immunogenic Poly-Epitope Frameshift Mutations in Microsatellite Unstable Tumors. Cell. 2020;183(6):1634-1649.e17.
145. Kloor M, Reuschenbach M, Pauligk C, Karbach J, Rafiyan M-R, Al-Batran S-E, et al. A Frameshift Peptide Neoantigen-Based Vaccine for Mismatch Repair-Deficient Cancers: A Phase I/IIa Clinical Trial. Clin Cancer Res. 2020;26(17):4503–10.
146. Leoni G, D'Alise AM, Cotugno G, Langone F, Garzia I, De

Lucia M, et al. A Genetic Vaccine Encoding Shared Cancer Neoantigens to Treat Tumors with Microsatellite Instability. Cancer Res. 2020;80(18):3972–82.
147. Ross JS, Fakih M, Ali SM, Elvin JA, Schrock AB, Suh J, et al. Targeting HER2 in colorectal cancer: The landscape of amplification and short variant mutations in ERBB2 and ERBB3. Cancer. 2018;124(7):1358–73.
148. Wang X-Y, Zheng Z-X, Sun Y, Bai Y-H, Shi Y-F, Zhou L-X, et al. Significance of HER2 protein expression and HER2 gene amplification in colorectal adenocarcinomas. World J Gastrointest Oncol. 2019;11(4):335–47.
149. Kruszewski WJ, Rzepko R, Ciesielski M, Szefel J, Zieliński J, Szajewski M, et al. Expression of HER2 in colorectal cancer does not correlate with prognosis. Dis Markers. 2010;29(5):207–12.
150. Bertotti A, Migliardi G, Galimi F, Sassi F, Torti D, Isella C, et al. A Molecularly Annotated Platform of Patient-Derived Xenografts ("Xenopatients") Identifies HER2 as an Effective Therapeutic Target in Cetuximab-Resistant Colorectal Cancer. Cancer Discov. 2011;1(6):508–23.
151. Sartore-Bianchi A, Amatu A, Porcu L, Ghezzi S, Lonardi S, Leone F, et al. HER2 Positivity Predicts Unresponsiveness to EGFR-Targeted Treatment in Metastatic Colorectal Cancer. Oncologist. 2019;24(10):1395–402.
152. Guarini C, Grassi T, Pezzicoli G, Porta C. Beyond RAS and BRAF: HER2, a New Actionable Oncotarget in Advanced Colorectal Cancer. Int J Mol Sci. 2021;22(13):6813.
153. Nakamura Y, Okamoto W, Kato T, Esaki T, Kato K, Komatsu Y, et al. Circulating tumor DNA-guided treatment with pertuzumab plus trastuzumab for HER2-amplified metastatic colorectal cancer: a phase 2 trial. Nat Med. 2021;27(11):1899–903.
154. Tosi F, Sartore-Bianchi A, Lonardi S, Amatu A, Leone F, Ghezzi S, et al. Long-term Clinical Outcome of Trastuzumab and Lapatinib for HER2-positive Metastatic Colorectal Cancer. Clin Colorectal Cancer. 2020;19(4):256-262.e2.
155. Sartore-Bianchi A, Lonardi S, Martino C, Fenocchio E, Tosi F, Ghezzi S, et al. Pertuzumab and trastuzumab emtansine in patients with HER2-amplified metastatic colorectal cancer: the phase II HERACLES-B trial. ESMO Open. 2020;5(5):e000911.
156. Meric-Bernstam F, Hurwitz H, Raghav KPS, McWilliams RR, Fakih M, VanderWalde A, et al. Pertuzumab plus trastuzumab for HER2-amplified metastatic colorectal cancer (MyPathway): an updated report from a multicentre, open-label, phase 2a, multiple basket study. Lancet Oncol. 2019;20(4):518–30.
157. Siena S, Elez E, Peeters M, André T, Van den Eynde M, Ng K, et al. PD-1 MOUNTAINEER: Open-label, phase 2 study of tucatinib combined with trastuzumab for HER2-positive metastatic colorectal cancer (SGNTUC-017, trial in progress). Ann Oncol. 2021;32:S199.
158. Siena S, Di Bartolomeo M, Raghav K, Masuishi T, Loupakis F, Kawakami H, et al. Trastuzumab deruxtecan (DS-8201) in patients with HER2-expressing metastatic colorectal cancer (DESTINY-CRC01): a multicentre, open-label, phase 2 trial. Lancet Oncol. 2021;22(6):779–89.
159. Ricarte-Filho JC, Li S, Garcia-Rendueles MER, Montero-Conde C, Voza F, Knauf JA, et al. Identification of kinase fusion oncogenes in post-Chernobyl radiation-induced thyroid cancers. J Clin Invest. 2013;123(11):4935–44.
160. Torre M, Vasudevaraja V, Serrano J, DeLorenzo M, Malinowski S, Blandin A-F, et al. Molecular and clinicopathologic features of gliomas harboring NTRK fusions. Acta Neuropathol Commun. 2020;8(1):107.
161. Farago AF, Taylor MS, Doebele RC, Zhu VW, Kummar S, Spira AI, et al. Clinicopathologic Features of Non-Small-Cell Lung Cancer Harboring an NTRK Gene Fusion. JCO Precis Oncol. 2018;2018.
162. Pietrantonio F, Di Nicolantonio F, Schrock AB, Lee J, Tejpar S, Sartore-Bianchi A, et al. ALK, ROS1, and NTRK Rearrangements in Metastatic Colorectal Cancer. J Natl Cancer Inst. 2017;109(12).
163. Cohen R, Pudlarz T, Delattre J-F, Colle R, André T. Molecular Targets for the Treatment of Metastatic Colorectal Cancer. Cancers (Basel). 2020;12(9):2350.
164. Martin-Zanca D, Hughes SH, Barbacid M. A human oncogene formed by the fusion of truncated tropomyosin and protein tyrosine kinase sequences. Nature. 319(6056):743–8.
165. Drilon A, Laetsch TW, Kummar S, DuBois SG, Lassen UN, Demetri GD, et al. Efficacy of Larotrectinib in TRK Fusion-Positive Cancers in Adults and Children. N Engl J Med. 2018;378(8):731–9.
166. Liu D, Offin M, Harnicar S, Li BT, Drilon A. Entrectinib: an orally available, selective tyrosine kinase inhibitor for the treatment of NTRK, ROS1, and ALK fusion-positive solid tumors. Ther Clin Risk Manag. 2018;Volume 14:1247–52.
167. Demetri GD, Paz-Ares L, Farago AF, Liu SV, Chawla SP, Tosi D, et al. Efficacy and safety of entrectinib in patients with NTRK fusion-positive (NTRK-fp) Tumors: Pooled analysis of STARTRK-2, STARTRK-1 and ALKA-372-001. Ann Oncol. 2018;29:viii713.
168. Drilon A, Nagasubramanian R, Blake JF, Ku N, Tuch BB, Ebata K, et al. A Next-Generation TRK Kinase Inhibitor Overcomes Acquired Resistance to Prior TRK Kinase Inhibition in Patients with TRK Fusion-Positive Solid Tumors. Cancer Discov. 2017;7(9):963–72.

Corresponding author:

Angelos Koutras Assoc. Prof.
Division of Oncology, Department of Medicine,
University Hospital of Patras, Patras, Greece, PC 26504,
Tel.: +30 2610999535, E-mail: angkoutr@upatras.gr

REVIEW ACHAIKI IATRIKI | 2022; 41(4):200–202

Acoustic biosensor: A brief review

Ariadni Konstantopoulou[1], Panagiota Balatli[2]

Abstract

Recently, the need for the rapid and accurate detection of different biological substances has led to the rapid development of a wide variety of biosensors. This has resulted to the development of biosensors and their applications across several domains, such as biotechnology, disease diagnosis, drug detection and food control. The technological progress of integrated reading circuits has led to the creation of small-scale bioremediation devices with high accuracy, fast response time and the ability to control real-time biomolecular interactions. Therefore, the heyday of electronic circuits and the design of detection devices contributes to the progress and the study of applications of interdisciplinary interest.

Key words: *Biosensor; Acoustic physics*

INTRODUCTION

A biosensor is a complete device that is independent and capable of displaying specific quantitative or semi-quantitative analytical information, using a biochemical receptor, i.e., a biological identifier that is in direct contact with a converter element. In 1969 the first potentiometric biosensor was built by Guilbault and Montalvo. Subsequently in 1980, the optical biosensors revolution began with the construction of the first fiber optic sensor for the in vivo detection of gases in the blood (Peterson). In 1983 the first surface plasmon resonance (SPR) immunosensor was produced and, in 1984 the first ammeter-mediated biosensor: ferrocene is used with glucose oxidase to detect glucose [1].

Analyzing the functional parts of a biosensor, we first encounter the bio-receptor which is integrated into a converter element, which converts the measured element into an output signal. A bio-receptor is a biological or biologically derived sensory element that typically uses certain types of molecules to identify biochemicals, such as enzymes, antibodies, nucleic acids, proteins, or a biological system (cells, tissues).

Thus, biosensors are divided based on the type of bio-receptor, into biogenic, where the bio-receptors have either antibodies or nucleic acid samples, and into biocatalysts, where the bio-receptors have a cell, enzyme, or tissue. Most transmission mechanisms are either optical or electrochemical based on mass, i.e., piezoelectric. In addition, electrochemical detection is based on the chemical properties of the specific substances which are present in a solution (analyzers) and are measured compared to a reference electrode. By selecting the mass-based detection method, the change in the frequency of the piezoelectric crystal, which depends on the mass of the crystal and the frequency of the applied electrical signal, provides useful information.

Biosensors are categorized based on the type of inverter in: electrochemical (ammeter, potentiometric, conductivity), optical (fiber optic, surface plasmon tuning, fluorescent), calorimetric (heat-conducting, isothermal, iso-environmental) and acoustic (surface acoustic wave, piezoelectric).

Acoustic Biosensors: Definition & Function

Electroacoustic biosensors function by detecting a change in mass density, due to the elastic, electrical or

[1]Department of Chemical Engineering
[2]Department of Material Science
University of Patras, Patras, Greece
Received: 31 Mar 2022; Accepted: 09 May 2022

dielectric properties of a membrane. This membrane consists of chemically interacting materials which are in contact with a piezoelectric material. In 1880 the Curie brothers discovered the piezoelectric effect, named after Henkel in 1881 and used in 1921 by Cady [2]. In piezoelectric sensors, when alternating voltage is applied, mechanical waves will be generated which will propagate through the substrate and will be converted back into an electric field which will be recorded as an electrical signal.

Exploiting elastic or acoustic waves is a modern challenge that could offer great opportunities for evolving technological applications [3]. Acoustic waves are longitudinal or transverse in the direction of their propagation and are characterized by surface or volume according to the dimensions of the instrument in which they propagate [4]. Acoustic sensors are divided into two categories depending on the type of wave they are operating. These are the BAW (volume wave based) acoustic sensors and the SAW surface acoustic sensors.

In acoustic volume sensors, the energy of the wave is diffused throughout the volume of the material to which it propagates, which is why these sensors are characterized by lower sensitivity. In contrast, in acoustic surface sensors, surface waves limit all their energy to the surface of the material to which they propagate, resulting in a higher sensitivity [4,5]. As the wave propagates, the surface of the sensor and any mass on it will oscillate simultaneously and thus an acoustic wave will be generated simultaneously. These changes depend on both the frequency at which the sensor oscillates and the type of the sensor. As a rule, increasing the operating frequency produces greater acoustic changes. When operating in an aquatic environment, the liquid in contact with the surface will oscillate following its movement, thus creating a damping acoustic field on the surface between liquid and solid (Figure 1) [6].

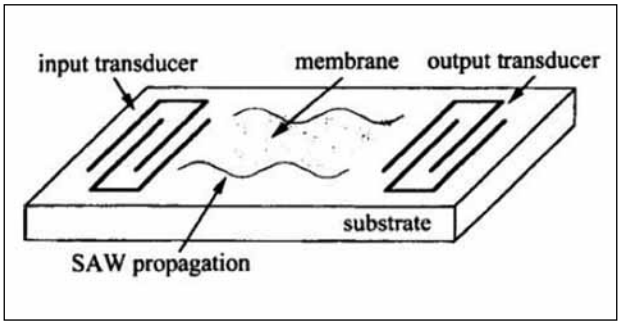

Figure 1. The propagation of sound waves on the surface of a sensor [6].

Applications

The need to study the interactions with other biological boundaries led to the creation of applications that involve the development of membrane bilayer models on surfaces. Other applications provide information on the kinetics of the interactions [7]. Recently, a class of Love type sensors was constructed which were designed to immobilize on their surface [8]. Tangibles specifically recognize a protein that is associated with blood clotting, thrombin and is a target for therapeutic agents.

Over the last decade, significant applications of piezoelectric biosensors have emerged, such as the reliable and rapid detection of the hepatitis B^9 virus. At the same time, there were cases of coupling of biosensors with other techniques such as atomic force microscopy, surface plasma tuning, and fluorescence microscopy in an attempt to increase their sensitivity and resolution [9].

In general, the number of commercially available instruments has increased significantly with the aim of developing sensors to study virtually any type of receptor-analyte interaction. The great challenge lies in the development of methods that combine biotechnology with the science of chemistry, biology, and physics for the simultaneous, multiple and needless labeling analysis of various bio-critical interactions in each area of modern research.

Conflict of interest disclosure: *None to declare.*

Declaration of funding sources: *None to declare.*

Author contributions: *Both authors performed the literature search, wrote the manuscript and approved the submitted version of the manuscript.*

REFERENCES

1. Reyes-De-Corcuera J, Cavalieri RP, Powers JR, Tang J, Kang DH. Enzyme-electropolymer-based amperometric biosensors: an innovative platform for time-temperature integrators. J Agric Food Chem. 2005;53(23):8866-73.
2. Ballato A. Piezoelectricity: history and new thrusts. IEEE Ultrasonics Symposium 1996; 1:575-83.
3. Konstantopoulou A, Aravantinos-Zafiris N, Sigalas MM. Acoustic Ring Biosensor. Rev Clin Pharmacol Pharmacokinet. 2019;(33):99-100.
4. Ballantine D, White R, Martin S, Rico A, Zellers E, Frye G, et

al. Acoustic Wave Sensors: Theory, Design and Physico-Chemical Applications. 1st ed. AP, San Diego; 1996.
5. Singh VR. Instrumentation techniques for acousto-optic studies in complex materials. Appl Acoust. 1990; 29(4):289-304.
6. Kress-Rogers E. Handbook of Biosensors and Electronic Noses. CRC Press Inc.: New York; 1997.
7. Harding GL, Du J, Dencher PR, Barnett D, Howe E. Love wave acoustic immunosensor operating in liquid. Sens Actuator A: Phys. 1997;(61): 279-86.
8. Schlensog MD, Gronewold T MA, Tewes M, Famulok M, Quandt E. A Love-wave biosensor using nucleic acids as ligands. Sens Actuators B: Chem. 2004; 101(3):308-15.
9. Zhou X, Liu L, Hu M, Wang L, Hu J. Detection of hepatitis B virus by piezoelectric biosensor. J Pharm Biomed. 2002;27(1-2):341-45.

Corresponding author:

Ariadni Konstantopoulou
Tel.: +30 6980800939
E-mail: ariadk@upatras.gr

The role of B cells in the pathogenesis of autoimmune diseases

Stamatis-Nick Liossis, Foteini Angelopoulou

Abstract

B lymphocytes are the effector cells of humoral immunity. Their multifaceted role spans from antibody and cytokine production to antigen presentation and T cell activation. Disturbances in their developmental pathways can have detrimental consequences as demonstrated by the complex molecular and clinical phenotypes of several autoimmune diseases. In recent years, the antibody-independent role of B cells in conditions classically thought of as T cell mediated has been supported by the successful implementation of B cell depletion therapies. Herein, we review the role of B cells in three autoimmune diseases: Systemic Lupus Erythematosus, Rheumatoid Arthritis and Systemic Sclerosis.

Key words: *B lymphocytes; Autoimmune diseases; Systemic sclerosis; Rheumatoid arthritis; Systemic lupus erythematosus.*

B CELL DEVELOPMENT AND MATURATION

The first indication of the existence of a cell population charged with antibody production was given by von Behring and Kitasato in 1890 when they remarked that circulating "antitoxins" where important for tetanus and diphtheria immunity [1]. It was nearly 80 years later that Max Cooper and Robert Good using a chicken animal model proved that cells derived from the bursa of Fabricius (the chicken equivalent of bone marrow) were responsible for antibody production while a different population of cells derived from the thymus mediated delayed-type hypersensitivity responses [2]. Since then, B lymphocytes have been the object of exciting research.

There are three well-characterized subpopulations of B cells. B1 cells that are produced in the fetal liver, have a distinct developmental process, self-renewing ability and are responsible for the majority of natural antibody production [3-5]. They have innate-like features and home mainly in the pleural and peritoneal cavity. B2 cells that are derived from multipotent hemopoietic stem cells in the bone marrow and subsequently migrate to secondary lymphoid organs (lymph nodes) where they mature and become activated via interaction with antigens. In 2002 a new B cell population was described, characterized as regulatory B cells, capable of producing the anti-inflammatory IL-10 [6]. This subset was able to suppress inflammatory responses in experimental murine models of collagen-induced arthritis and autoimmune encephalitis [7,8]. A critical step in B cell development is the B cell receptor generation. This is achieved by a complex process involving continuous gene segment rearrangement of the Ig heavy and light chain loci. B cells have the ability during their development to rearrange the V, D and J gene segments in the heavy chain locus and V, J gene segments in the light chain locus [9-11]. This mechanism is utilized in class switching and affinity maturation of B cell receptors. Antigen receptor gene rearrangement ensures diversity in the receptor repertoire of B cells and therefore sufficient immunity against a variety of different pathogens, such as viruses, bacteria etc. It is also one of the mechanisms employed to avert self-reactive cells. Taking into account the wide variety of the produced antigenic receptors that guarantee an adequate immune response, it is only logical and statistically probable that a portion of them would recognize self-antigens. In order to avert self-reactivity a network of sophisticated mechanisms is employed for the silencing of self-reactive B cells.

Department of Rheumatology, University of Patras Medical School and Patras University Hospital, Patras, Greece
Received: 09 Apr 2022; Accepted: 03 Jun 2022

Self-reactive B cells can be silenced either during their development in the bone marrow (central tolerance), or during their maturation and activation in peripheral lymphoid organs (peripheral tolerance). Central tolerance is ensured by three mechanisms: Clonal deletion, clonal anergy and receptor editing. The strong recognition of a self-antigen present at a high concentration in the bone marrow induces either cellular death by apoptosis (clonal deletion) or a light chain recombination resulting in a new, possibly not self-reactive receptor (receptor editing). The inability to exert effector functions (clonal anergy) is induced by BCR activation without concurrent co-stimulatory receptor activation. About 20% of moderately auto-reactive B cells still manage to migrate to the periphery, where they become anergic or undergo cellular death (apoptosis) [12,13]. When immune tolerance is breached, interaction with the correspondent self-antigens initiates an inflammatory response and its consequences, destruction of healthy tissues and organs. B cells are the driving force in five pathological processes involved in autoimmunity: autoantibody production, processing and presentation of autoantigens to autoreactive T-cells, inflammatory cytokine production and development of ectopic tertiary lymphoid structures.

In this paper we aim to summarize the direct and indirect role of B cells in three autoimmune disorders: systemic lupus erythematosus, rheumatoid arthritis and systemic sclerosis.

SYSTEMIC LUPUS ERYTHEMATOSUS

Systemic Lupus Erythematosus (SLE) is considered as the model for systemic autoimmunity. It is a multifactorial disease associated with significant morbidity and mortality and up today presents a therapeutic challenge for the practicing physician. It primarily affects women of child-bearing age, of Hispanic and African American ancestry, and its clinical presentation can be highly heterogenous, affecting multiple systems [14]. Despite its varying phenotype and multiple hypothesized pathophysiological pathways involved, intrinsic B cell dysregulation appears to be a common denominator in both human and animal models.

The extensive repertoire of (auto) antibodies against a multitude of self-antigens is a hallmark of the condition, with antinuclear antibodies being an almost universal characteristic [15-17]. Of note, anti-dsDNA and anti-Sm antibodies to this day remain part of the ACR criteria for SLE diagnosis [18]. A portion of these autoantibodies exert a well-established pathogenetic role and are directly linked to specific clinical manifestations and phenotypes of the disease.

Autoantibodies against antigens of red blood cells may cause autoimmune hemolytic anemia [19,20]. Certain anti-DNA antibodies cross react with NMDA receptors in the central nervous system and are able to breach the blood-brain barrier causing neuronal cell death in murine models [21]. In SLE patients, anti-NMDA-R antibodies have been associated with neurocognitive defects [22]. When anti-Ro antibodies are present in the sera of pregnant SLE patients, they can cause congenital heart block in approximately 2% of neonates [23]. Antibodies against phospholipids and β2-glycoprotein are associated with thrombotic events and recurrent miscarriages, a clinical entity known as antiphospholipid syndrome [24].

Autoantibodies also yield their noxious capacity via the formation and deposition of immune complexes. One of the most severe and life-threatening manifestations of SLE, lupus nephritis, could be instigated by the deposition of immune complexes and subsequent inflammatory cascade and tissue injury [25-27]. While evidence to fully delineate the multifaceted role of B cells in human SLE is circumstantial and occasionally contradictory, animal model studies provide considerably more consistent results. Murine models particularly have been crucial in underscoring the antibody-independent functions of B cells in autoimmunity. MRL/lpr mice are a model for systemic autoimmunity with a clinical phenotype resembling SLE and similar antibody profile, with anti-ds DNA antibodies. In 1994, Schlomchik et al proved that when made B cell deficient, these mice did not exhibit any sign of autoimmune glomerulonephritis or vasculitis, and as expected, lacked autoantibodies. Additionally, T cell activation was remarkably diminished, highlighting the necessity of B cell-T cell interaction for optimal cellular immune responses [28].

Chan et al later used the same murine model to demonstrate that when B cells were genetically engineered to express surface Ig but not secret soluble immunoglobulins, disease expression was not attenuated, with the mice developing severe nephritis and vasculitis, providing further proof that antibody-independent B cell functions contribute to disease expression [29]. In an attempt to further elucidate the mechanisms behind the breach of immune tolerance, characterization of the surface molecular phenotype of B lymphocytes became of great interest, thus providing invaluable information

about peripheral blood composition of B cell subsets. Firstly, there seems to be an expansion of plasma and memory B cell compartments, while peripheral naive B cells are consistently reduced [30-33]. Circulating plasma cell levels have also been strongly associated with disease activity and anti-dsDNA antibody titers [34]. Of note, the FcγRIIb inhibitory receptor is underexpressed in SLE memory B cells, leading to a lower threshold of reactivation and ensuing differentiation into antibody secreting cells [35].

Regulatory B cells have also been found functionally impaired, unable to suppress T helper cell proliferation in SLE. While murine models suggest reduced levels of IL-10 as the resulting malfunction of regulatory B cell impairment [36,37], human studies indicate that IL-10 enhances rather than suppresses disease expression, thus creating yet another conundrum [38-40].

A recently discovered subset of B cells, referred to as Age Related B cells (ABCs) has been the focus of great interest. ABCs were initially thought of as an antigen-experienced, exhausted phenotype associated with normal senescence and inflammation such as autoimmunity and infections [41-44]. Exhaustive studies have further classified the nature of these cells, depending on surface marker expression and transcriptional signatures. Specifically, CD11hiTbet+ B cells, a population expanded in SLE but not healthy elders, shares many of ABCs key features, such as their antigen presentation capacity and their ability to differentiate into antibody secreting cells [45,46]. Interestingly, the expansion of this population has also been associated with increased disease activity scores and antinuclear antibody titers [45]. Further studies are required to illuminate their cryptic role, origin and developmental pathways.

The immunopathogenic role of B-cell derived cytokines has also been extensively studied in lupus, offering useful insights into the complex pathways leading to the breach of self-tolerance. Aberrant IL-6 production by lupus B cells drives their terminal differentiation into antibody secreting cells and induces further IL-6 production, creating a positive feedback loop [47]. Supporting this finding, IL-6 receptor blockade significantly suppresses auto-antibody secretion in human lupus B cells, while lupus-prone mice treated with anti-IL-6 monoclonal antibodies display substantially mitigated kidney disease and antibody production [48]. IL-4, IL-21, IFN-γ, TGF-β and lymphotoxin α produced by B cells also exert pathogenic roles in the propagation of inflammation and tissue injury [49,50].

The pivotal role of B cell dysregulation in the cellular and molecular events leading to the eventual presentation of lupus is showcased by the central role of B depletion therapies in our therapeutic armamentarium in recent years. Despite the failure of large clinical trials to exhibit therapeutic effect [51], Rituximab, an anti-CD20 monoclonal antibody is, to this day, successfully used in life-threatening presentations and refractory disease. Further investigation into the aberrant developmental pathways and infringement on self-tolerance checkpoints of B cells is required in order to guide individualized treatment and minimize adverse events.

SYSTEMIC SCLEROSIS

Systemic sclerosis (SSc) is an heterogenous systemic autoimmune disease characterized by the excessive production and deposition of extracellular matrix in the skin and visceral organs such as the oesophagus, lower gastrointestinal track, heart and lungs, resulting in phenotypic variation [52]. Autoantibodies is a central feature of the disease, with antinuclear antibodies being present in >90% of patients [53]. Hypergammaglobulinemia and polyclonal B cell hyperactivity have also been well documented laboratory characteristics of SSc patients [54]. A direct link between autoimmunity and fibrosis, however, is yet to be discovered. Autoantibodies associated with SSc include anti-topoisomerase I abs, anti -RNA polymerase abs, anticentromere antibodies or anti-Th/To Ab [55,56]. However, autoantibodies have not been directly linked to fibrosis. This finding is supported by the two most accurate mouse models for SSc at our disposal, the tight skin (TSK/+) mouse and the Bleomycin (BLM) treated mouse. The tight skin mouse is the product of a tandem mutation in the fibrillin-1 gene resulting in a fibrotic phenotype resembling SSc, with subcutaneous inflammatory infiltration and fibrosis and lung emphysema [57].

Bleomycin is an antibiotic used widely as a chemotherapeutic agent that, when injected subcutaneously in mice, causes lung fibrosis, dermal inflammatory infiltration and fibrosis as well as autoantibody production [58]. A recently described feature of SSc is the expansion of naive B cell populations while memory B cells as well as plasmablasts/ plasma cell precursor populations are diminished due to enhanced apoptosis. However, despite their reduced number, SSc memory B lymphocytes were found chronically activated in vivo, with an increased capacity to produce autoantibodies [59].

B regulatory cells, a subpopulation that produces

the anti-inflammatory IL-10, were also shrinked, and serum IL-10 levels reduced compared to healthy controls [60]. Another well described phenotypic change in peripheral SSc B cells is the over expression and increased phosphorylation of the positive signal modulator CD19 [61,62]. CD19 is a transmembrane protein widely expressed in all stages of B cell development that can decrease the threshold for BCR activation [63]. When CD19 expression was studied in TSK/+ mice it was found unaltered compared to wild type mice. However, CD19 phosphorylation was indeed increased and CD19 mediated intracellular signaling was enhanced early in B cell activation leading to the conclusion that TSK/+ mice B cells are indeed chronically activated [64,65]. Additionally, CD19 loss in TSK/+ completely abrogated hypergammaglobulinemia and autoantibody production, while it ameliorated skin fibrosis [64]. Additionally, CD22, a negative regulatory molecule that increases the threshold for B cell activation preventing aberrant immune responses, is under-expressed in SSc B cells [65].

Cytokine production, another important capacity, also appears to be impaired in systemic sclerosis, with overwhelming evidence supporting the overproduction of pro-inflammatory, profibrogenic cytokines such as IL-6 and TGF-b. Increase of the pro-inflammatory IL-6 in the sera of patients with systemic sclerosis, compared to healthy controls has also been well documented and is associated with the extend of dermal fibrosis [66,67]. Finally, direct B cell-fibroblast interaction has also been described. It has been proven by in vitro experiments, that direct B cell-fibroblast contact is needed for the stimulation of fibroblasts and the excess production of collagen. Lastly, the effectiveness of B cell depletion therapies in the treatment of systemic sclerosis, especially lung involvement, is a testament to the true extent of B cell involvement in SSc pathogenesis [68].

RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is an autoimmune disease characterized by symmetric inflammatory polyarthritis leading to progressive bone and cartilage erosion, that can often be accompanied by extra-articular manifestations, such as subcutaneous rheumatoid nodules, sicca syndrome, peripheral neuropathy and pulmonary interstitial fibrosis. Its pathogenesis is complex and driven by many simultaneous pathological processes [69]. Many immune cells such as T lymphocytes, macrophages and B cells are involved in the synovial inflammatory process.

B cells were first implicated in the pathogenesis of RA as the producers of the autoantibodies RF and anti-CCP, that have served as excellent diagnostic and predictive biomarkers [70]. Rheumatoid factor is an autoantibody against the Fc portion of IgG and anti-CCPs are directed against peptides and proteins that have been post-translationally modified by a process called citrullination. Citrullination is the conversion of arginine into citrulline by a cluster of enzymes called arginine deiminases [71]. Although RF is not exclusive to RA and can present in other autoimmune diseases, infections as well as approximately 10% of healthy individuals, high titers of RF have been associated with clinically and radiographically more severe disease, functional impairment and extra-articular manifestations [72]. A wide variety of other autoantibodies have since been associated with RA including but not limited to antibodies against type II collagen, immunoglobulin-binding protein, rheumatoid arthritis- associated autoantigen hnRNP-A2 (RA33), glucose-6- phosphate isomerase (GPI), and calp statin [73-76]. Despite the fact that their contribution to synovial inflammation has been elegantly demonstrated in animal models, such as the collagen-induced arthritis murine model and the K/B-N model, it still remains unclear in human disease [77].

As we have already mentioned, B cells serve as excellent antigen-presenting cells, especially in low-concentration antigens. Specifically in RA, RF+ B cells take up RF-containing immunocomplexes via their antigenic receptors and proceed to process and present antigens to T helper cells, thus orchestrating a stronger and more effective adaptive immune response [78]. B cells are also thought to be crucial for synovial lymphoneogenesis. Ectopic follicle-like synovial structures rich in B cells, T cells, macrophages and FDCs with a follicle-like architecture and germinal centers have been found in approximately 25% of RA patients. Takemura et al studied synovial biopsies from RA patients undergoing joint replacement surgery [79]. They were able to distinguish three distinct patterns: diffuse infiltration of B cells, T cells, dendritic cells and macrophages lacking structure, B- and T-cell infiltrates resembling secondary follicles with germinal centers and B-, T- cell aggregates lacking germinal centers. Furthermore, they found actively proliferating B cells and follicular dendritic cell networks only in the aggregates containing germinal centers. Similarly, Humby et al also found three histological patterns a lympho-myeloid pattern with a B cell predominance, a diffuse myeloid-lineage infiltration pattern with low B cell counts and a pauci-immune with

Table 1. *B cell aberrations in autoimmune diseases.*

	Systemic Lupus Erythematosus	Systemic Sclerosis	Rheumatoid Arthritis
Hyperactivated phenotype	Under expression of inhibitory FcγRII receptor in memory B cells, functionally impaired Bregs, Increased plasma- secreting differentiation capacity of ABCs	Increased expression of positive signal modulator CD19, underexpression of negative signal modulator CD22, reduced Bregs	*
Auto-antibody production	ANA, anti-dsDNA, anti-NMDA-R, anti-Ro, anti-La, anti-β2glycoprotein, anticardiolipin antibodies	anti-Topoisomerase I, anti-Polymerase, anticentromere, anti-Th/To antibodies	Antibodies against type II collagen, immunoglobulin binding protein, GPI, calp statin, hnRNP-A2(RA33)
Processing of antigens Antigen presentation to auto-reactive T cells	B cell subset with Increased antigen-presenting capacity (Age-Related B cells)	* *	Uptake, processing and subsequent antigen presentation of RF-immunocomplexes by RF+ B cells
Inflammatory production cytokine	Increased B-cell derived IL-6, IL-4,IL-21,IFN-γ,TGF-β	Overproduction of Proinflammatory IL-6, TGF-β	Increased B cell derived proinflammatory IL-6, lymphotoxin β
Ectopic tertiary lymphoid structure development	*	*	Presence of follicle-like infiltrates rich in B- and T- cells in RA patients' synovium

ABCs: Age associated B cells
*not sufficient evidence

a prevalent stromal cell presence [80]. In addition to the aforementioned autoantibody production, antigen presentation and contribution to lymph neogenesis, B cells also produce cytokines that propagate the inflammatory process such IL-6, IL-10 and lymphotoxin β, providing a positive reinforcement circuit for optimal T-cell/macrophage activation [81]. Although, their role is not fully elucidated, the successful treatment of RA with B cell depletion therapies is a strong indicator that B cells are one of the driving forces behind the aberrant synovial immune response.

CONCLUSION

B cells were brought to the spotlight as the producer cells of antibodies. Their role however, proved to be substantially more intricate. That is masterfully demonstrated by the catastrophic results of the disruption of their homeostasis (Table 1). Their functional repertoire spans from antigen presentation and T cell activation to pro- and anti-inflammatory cytokine production. Their study continues to yield exciting new results. B lymphocyte dysregulation has been discovered in classically considered T cell mediated autoimmune diseases, something that is highlighted by the successful implementation of B cell depletion therapies.

Conflict of interest disclosure: *None to declare*

Declaration of funding sources: *None to declare*

Author Contributions: *FA performed the literature search, extracted data from the retrieved articles, analyzed the data and participated in manuscript drafting; SNL conceived the idea and designed the study, extracted data from the retrieved articles, analyzed the data and participated in manuscript drafting. Both authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.*

REFERENCES

1. Behring E, Kitasato S. Über das Zustandekommen der Diphtherie- Immunität und der Tetanus-Immunität bei Thieren. Dtsch Med Wochenschrift. 1890;49:1113-4.
2. Cooper M, Peterson R, Good R. Delineation of the Thymic and Bursal Lymphoid Systems in the Chicken. Nature 1965;205:143–6.
3. Hayakawa K, Hardy RR, Herzenberg LA. Progenitors for Ly-1 B cells are distinct from progenitors for other B cells. J Exp Med. 1985;161(6):1554–68.
4. Solvason N, Lehuen A, Kearney JF. An embryonic source of Ly1 but not conventional B cells. Int Immunol. 1991;3(6):543–50.

5. Clarke SH, Arnold LW. B-1 cell development: evidence for an uncommitted immunoglobulin (Ig)M+ B cell precursor in B-1 cell differentiation. J Exp Med. 1998;187(8):1325–34.
6. Mizoguchi A, Mizoguchi E, Takedatsu H, Blumberg RS, Bhan AK. Chronic intestinal inflammatory condition generates IL-10-producing regulatory B cell subset characterized by CD1d upregulation. Immunity. 2002;16(2):219-30.
7. Fillatreau S, Sweenie CH, McGeachy MJ, Gray D, Anderton SM. B cells regulate autoimmunity by provision of IL-10. Nat Immunol. 2002;3(10):944-50.
8. Mauri C, Gray D, Mushtaq N, Londei M. Prevention of arthritis by interleukin 10-producing B cells. J Exp Med. 2003;197(4):489-501.
9. Komori T, Okada A, Stewart V, Alt FW. Lack of N regions in antigen receptor variable region genes of TdT-deficient lymphocytes. Science. 1993;261(5125):1171-5.
10. Tonegawa S. Somatic generation of antibody diversity. Nature. 1983;302(5909):575-81.
11. Tobón GJ, Izquierdo JH, Cañas CA. B lymphocytes: development, tolerance, and their role in autoimmunity-focus on systemic lupus erythematosus. Autoimmune Dis. 2013;2013:827254.
12. Abbas, Abul K., Andrew H. Lichtman, and Shiv Pillai. Cellular and Molecular Immunology. 6th ed. Philadelphia: Saunders Elsevier, 2007.
13. Nossal GJ. Cellular mechanisms of immunologic tolerance. Annu Rev Immunol. 1983;1(1):33-62.
14. Duarte C, Couto M, Ines L, Liang MH. Epidemiology of systemic lupus erythematosus. In: Lahita RG, Tsokos G, Buyon J, Koike T, eds. Systemic lupus erythematosus. 5th ed. London: Elsevier, 2011:673-96.
15. Tsokos GC, Kammer GM. Molecular aberrations in human systemic lupus erythematosus. Mol Med Today 2000;6(11):418-24.
16. Conrad K, Roggenbuck D, Reinhold D, Sack U. Autoantibody diagnostics in clinical practice. Autoimmun. Rev. 2012;11(3):207–11.
17. Fritzler MJ. Clinical relevance of autoantibodies in systemic rheumatic diseases. Mol Biol Rep 1996;23(3-4):133–45.
18. Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982;25(11):1271-7.
19. Victoria EJ, Pierce SW, Branks MJ, Masouredis SP. IgG red blood cell autoantibodies in autoimmune hemolytic anemia bind to epitopes on red blood cell membrane band 3 glycoprotein. J Lab Clin Med. 1990;115(1):74-88.
20. Jeffries M, Hamadeh F, Aberle T, Glenn S, Kamen DL, Kelly JA, et al. Haemolytic anaemia in a multi-ethnic cohort of lupus patients: a clinical and serological perspective. Lupus. 2008;17(8):739-43.
21. Wang X, Ma C, Liu CY, Li GJ, Zhao D, Han DF. Neuronal NMDAR Currents of the Hippocampus and Learning Performance in Autoimmune Anti-NMDAR Encephalitis and Involvement of TNF-α and IL-6. Front Neurol. 2019;10:684.
22. DeGiorgio LA, Konstantinov KN, Lee SC, Hardin JA, Volpe BT, Diamond B. A subset of lupus anti-DNA antibodies cross-reacts with the NR2 glutamate receptor in systemic lupus erythematosus. Nat Med. 2001;7(11):1189-93.
23. Brucato A, Cimaz R, Caporali R, Ramoni V, Buyon J. Pregnancy outcomes in patients with autoimmune diseases and anti-Ro/SSA antibodies. Clin Rev Allergy Immunol 2011;40(1):27-41.
24. Ruiz-Irastorza G, Crowther M, Branch W, Khamashta MA. Antiphospholipid syndrome. Lancet 2010;(9751):1498-509.
25. Kimberly RP. Immune complexes in the rheumatic diseases. Rheum Dis Clin North Am 1987;13(3):583-96.
26. Chang A, Henderson SG, Brandt D, Liu N, Guttikonda R, Hsiehet C, al. In situ B cell-mediated immune responses and tubulointerstitial inflammation in human lupus nephritis. J Immunol 2011;186(3):1849-60.
27. Mayadas TN, Tsokos GC, Tsuboi N. Mechanisms of immune complex-mediated neutrophil recruitment and tissue injury. Circulation 2009;120(20):2012-24.
28. Shlomchik MJ, Madaio MP, Ni D, Trounstein M, Huszar D. The role of B cells in lpr/lpr-induced autoimmunity. J Exp Med. 1994;180(4):1295-306.
29. Chan OT, Hannum LG, Haberman AM, Madaio MP, Shlomchik MJ. A novel mouse with B cells but lacking serum antibody reveals an antibody-independent role for B cells in murine lupus. J Exp Med 1999;189:1639–48.
30. Odendahl M, Jacobi A, Hansen A, et al. Disturbed peripheral B lymphocyte homeostasis in systemic lupus erythematosus. J Immunol 2000;165(10):5970-9.
31. Dörner T, Jacobi AM, Lipsky PE. B cells in autoimmunity. Arthritis Res Ther. 2009;11(5):247.
32. Fecteau JF, Côté G, Néron S. A new memory CD27-IgG+ B cell population in peripheral blood expressing VH genes with low frequency of somatic mutation. J Immunol. 2006;177(6):3728-36.
33. Jacobi AM, Reiter K, Mackay M, Aranow C, Hiepe F, Radbruch A, et al. Activated memory B cell subsets correlate with disease activity in systemic lupus erythematosus: delineation by expression of CD27, IgD, and CD95. Arthritis Rheum. 2008 ;58(6):1762-73.
34. Jacobi AM, Odendahl M, Reiter K, Bruns A, Burmester GR, Radbruch A et al. Correlation between circulating CD27high plasma cells and disease activity in patients with systemic lupus erythematosus. Arthritis Rheum. 2003;48(5):1332-42.
35. Mackay M, Stanevsky A, Wang T, Aranow C, Li M, Koenig S, et al. Selective dysregulation of the FcgammaIIB receptor on memory B cells in SLE. J Exp Med. 2006 4;203(9):2157-64.
36. Watanabe R, Ishiura N, Nakashima H, Kuwano Y, Okochi H, Tamaki K, et al. Regulatory B cells (B10 cells) have a suppressive role in murine lupus: CD19 and B10 cell deficiency exacerbates systemic autoimmunity. J Immunol. 2010;184(9):4801-9.
37. Yin Z, Bahtiyar G, Zhang N, Liu L, Zhu P, Robert ME, et al. IL-10 regulates murine lupus. J Immunol. 2002;169(4):2148-55.
38. Pathak S, Mohan C. Cellular and molecular pathogenesis of systemic lupus erythematosus: lessons from animal models. Arthritis Res Ther. 2011;13(5):241.

39. E. R. Capper, J. K. Maskill, C. Gordon, and A. I. F. Blakemore, Interleukin (IL)-10, IL-1ra and IL-12 proles in active and quiescent systemic lupus erythematosus: could longitudinal studies reveal patient subgroups of differing pathology? Clin. Exp. Immunol 2004;138(2):348-56.
40. Tyrrell-Price J, Lydyard PM, Isenberg DA. The effect of interleukin-10 and of interleukin-12 on the in vitro production of anti-double-stranded DNA antibodies from patients with systemic lupus erythematosus. Clin Exp Immunol. 2001;124(1):118-25.
41. Hao Y, O'Neill P, Naradikian MS, Scholz JL, Cancro MP. A B-cell subset uniquely responsive to innate stimuli accumulates in aged mice. Blood.2011;118(5):1294-304.
42. Rubtsov AV, Rubtsova K, Fischer A, Meehan RT, Gillis JZ, Kappler JW, et al. Toll-like receptor 7 (TLR7)-driven accumulation of a novel CD11c+ B-cell population is important for the development of autoimmunity. Blood. 2011;118(5):1305-15.
43. Weiss GE, Crompton PD, Li S, Walsh LA, Moir S, Traore B, et al. Atypical memory B cells are greatly expanded in individuals living in a malaria-endemic area. J Immunol 2009;183(3):2176-82.
44. Moir S, Ho J, Malaspina A, Wang W, DiPoto AC, O'Shea MA, et al. Evidence for HIV-associated B cell exhaustion in a dysfunctional memory B cell compartment in HIV-infected viremic individuals. J Exp Med. 2008;205(8):1797-805.
45. Wang S, Wang J, Kumar V, Karnell JL, Naiman B, Gross PS, et al. IL-21 drives expansion and plasma cell differentiation of autoreactive CD11chiT-bet+ B cells in SLE. Nat Commun. 2018;9(1):1758.
46. Sachinidis A, Xanthopoulos K, Garyfallos A. Age-Associated B Cells (ABCs) in the Prognosis, Diagnosis and Therapy of Systemic Lupus Erythematosus (SLE). Mediterr J Rheumatol. 2020;31(3):311-18.
47. Kitani A, Hara M, Hirose T, Harigai M, Suzuki K, Kawakami M, et al. Autostimulatory effects of IL-6 on excessive B cell differentiation in patients with systemic lupus erythematosus: analysis of IL-6 production and IL-6R expression. Clin Exp Immunol. 1992;88(1):75-83.
48. Liang B, Gardner DB, Griswold DE, Bugelski PJ, Song XY. Anti-interleukin-6 monoclonal antibody inhibits autoimmune responses in a murine model of systemic lupus erythematosus. Immunology. 2006;119(3):296-305.
49. Youinou P, Taher TE, Pers JO, Mageed RA, Renaudineau Y. B-lymphocyte cytokines and rheumatic autoimmune disease. Arthritis Rheum. 2009;60(7):1873-80.
50. Ohl K, Tenbrock K. Inflammatory cytokines in systemic lupus erythematosus. J Biomed Biotechnol. 2011;2011:432595.
51. Merrill JT, Neuwelt CM, Wallace DJ, Shanahan JC, Latinis KM, Oates JC, et al. Efficacy and safety of rituximab in moderately-to-severely active systemic lupus erythematosus: the randomized, double-blind, phase II/III systemic lupus erythematosus evaluation of rituximab trial. Arthritis Rheum. 2010;62(1):222-33.
52. Denton CP, Khanna D. Systemic sclerosis. Lancet. 2017;390(10103):1685-99.
53. Okano Y. Antinuclear antibody in systemic sclerosis (scleroderma). Rheum Dis Clin North Am. 1996;22(4):709-35.
54. Famularo G, Giacomelli R, Alesse E, Cifone MG, Morrone S, Boirivant M, et al. Polyclonal B lymphocyte activation in progressive systemic sclerosis. J Clin Lab Immunol. 1989;29(2):59-63.
55. Ceribelli A, Cavazzana I, Franceschini F, Airò P, Tincani A, Cattaneo R, et al. Anti-Th/To are common antinucleolar autoantibodies in Italian patients with scleroderma. J Rheumatol. 2010;37(10):2071-5.
56. Duarte A, Cordeiro A, Santos M, J. da Silva C. Antibody profile and systemic sclerosis clinical features – myth or reality? Ann Rheum Dis. 2018;77:1502-3.
57. Pablos JL, Everett ET, Norris JS. The tight skin mouse: an animal model of systemic sclerosis. Clin Exp Rheumatol. 2004;22(3 Suppl 33):81-5.
58. Liang M, Lv J, Zou L, Yang W, Xiong Y, Chen X, et al. A modified murine model of systemic sclerosis: bleomycin given by pump infusion induced skin and pulmonary inflammation and fibrosis. Lab Invest. 2015;95(3):342-50.
59. Sato S, Fujimoto M, Hasegawa M, Takehara K. Altered blood B lymphocyte homeostasis in systemic sclerosis: expanded naive B cells and diminished but activated memory B cells. Arthritis Rheum. 2004;50(6):1918-27.
60. Matsushita T, Hamaguchi Y, Hasegawa M, Takehara K, Fujimoto M. Decreased levels of regulatory B cells in patients with systemic sclerosis: association with autoantibody production and disease activity. Rheumatology (Oxford). 2016 ;55(2):263-7.
61. Sato S, Hasegawa M, Fujimoto M, Tedder TF, Takehara K. Quantitative genetic variation in CD19 expression correlates with autoimmunity. J Immunol. 2000;165(11):6635-43.
62. Soto L, Ferrier A, Aravena O, Fonseca E, Berendsen J, Biere A, et al. Systemic Sclerosis Patients Present Alterations in the Expression of Molecules Involved in B-Cell Regulation. Front Immunol. 2015;6:496.
63. Del Nagro CJ, Otero DC, Anzelon AN, Omori SA, Kolla RV, Rickert RC. CD19 function in central and peripheral B-cell development. Immunol Res. 2005;31(2):119-31.
64. Saito E, Fujimoto M, Hasegawa M, Komura K, Hamaguchi Y, Kaburagi Y, et al. CD19-dependent B lymphocyte signaling thresholds influence skin fibrosis and autoimmunity in the tight-skin mouse. J Clin Invest. 2002;109(11):1453-62.
65. Asano N, Fujimoto M, Yazawa N, Shirasawa S, Hasegawa M, Okochi H, et al. B Lymphocyte signaling established by the CD19/CD22 loop regulates autoimmunity in the tight-skin mouse. Am J Pathol. 2004;165(2):641-50.
66. Hasegawa M, Sato S, Ihn H, Takehara K. Enhanced production of interleukin-6 (IL-6), oncostatin M and soluble IL-6 receptor by cultured peripheral blood mononuclear cells from patients with systemic sclerosis. Rheumatology (Oxford). 1999 ;38(7):612-7.
67. Sato S, Hasegawa M, Takehara K. Serum levels of interleukin-6 and interleukin-10 correlate with total skin thickness score in patients with systemic sclerosis. J Dermatol Sci 2001;27(2):140–6.
68. Goswami RP, Ray A, Chatterjee M, Mukherjee A, Sircar G,

Ghosh P. Rituximab in the treatment of systemic sclerosis-related interstitial lung disease: a systematic review and meta-analysis. Rheumatology (Oxford). 2021;60(2):557-67.
69. Grassi W, De Angelis R, Lamanna G, Cervini C. The clinical features of rheumatoid arthritis. Eur J Radiol. 1998;27(Suppl 1):18-24.
70. Song YW, Kang EH. Autoantibodies in rheumatoid arthritis: rheumatoid factors and anticitrullinated protein antibodies. QJM. 2010;103(3):139-46.
71. Makrygiannakis D, af Klint E, Lundberg IE, Löfberg R, Ulfgren AK, Klareskog L, et al. Citrullination is an inflammation-dependent process. Ann Rheum Dis. 2006;65(9):1219-22.
72. Graudal N. The natural history and prognosis of rheumatoid arthritis: association of radiographic outcome with process variables, joint motion and immune proteins. Scand J Rheumatol. 2004;33(Suppl118):1-37.
73. Mewar D, Wilson AG: Autoantibodies in rheumatoid arthritis: a review. Biomed Pharmacother. 2006;60(10):648–55.
74. Hoffmann MH, Tuncel J, Skriner K, Tohidast-Akrad M, Türk B, Pinol-Roma S, et al. The rheumatoid arthritis-associated autoantigen hnRNP-A2 (RA33) is a major stimulator of autoimmunity in rats with pristane-induced arthritis. J Immunol 2007;179(1):7568–76.
75. Furuya T, Matsumoto I, Tsuchiya N, Hakoda M, Ichikawa N, Yago T, et al. Anti-glucose-6-phosphate isomerase, anti-cyclic citrullinated peptide antibodies and HLA- DRB1 genotypes in Japanese patients with early rheumatoid arthritis. Clin Exp Rheumatol 2008;26(5):918–21.
76. Auger I, Roudier C, Guis S, Balandraud N, Roudier J. HLA-DRB1*0404 is strongly as- sociated with anticalpastatin antibodies in rheumatoid arthritis. Ann Rheum Dis 2007;66(12):1588–93.
77. Bessis N, Decker P, Assier E, Semerano L, Boissier MC. Arthritis models: usefulness and interpretation. Semin Immunopathol. 2017;39(4):469-486.
78. Roosnek E, Lanzavecchia A. Efficient and selective presentation of antigen-antibody complexes by rheumatoid factor B cells. J Exp Med. 1991;173(2):487-9.
79. Takemura S, Braun A, Crowson C, Kurtin PJ, Cofield RH, O'Fallon WM, et al. Lymphoid neogenesis in rheumatoid synovitis. J Immunol. 2001 15;167(2):1072-80.
80. Humby F, Lewis M, Ramamoorthi N, Hackney JA, Barnes MR, Bombardieri M, et al. Synovial cellular and molecular signatures stratify clinical response to csDMARD therapy and predict radiographic progression in early rheumatoid arthritis patients. Ann Rheum Dis. 2019;78(6):761-72.
81. McInnes IB, Schett G. Cytokines in the pathogenesis of rheumatoid arthritis. Nat Rev Immunol. 2007;7(6):429-42.

Corresponding author:

Stamatis-Nick Liossis
Department of Internal Medicine, Division of Rheumatology, University of Patras Medical School, 26504, Rion, Patras, Greece
Tel.: +30 2613 603 693, Fax: +30 2610-993 982
E-mail: snliossis@med.upatras.gr

Treatment evolution of Systemic Lupus Erythematosus

Chrysanthi Staveri

Abstract

Systemic Lupus Erythematosus (SLE) is a chronic multisystem autoimmune disease with diverse clinical manifestations ranging from mild to severe or even life-threatening. The purpose of this review is to summarize data on SLE management that have emerged over the last few years. Regimens that have been investigated target B cells, plasma cells, T cells and plasmacytoid dendritic cells. Additionally, treatments that affect B cell survival and specific intracellular pathways such as the JAK STAT pathway, the IFN pathway and mTOR or cytokines have also been used in the treatment of SLE or are currently being evaluated. Despite the existence of several therapeutic regimens in SLE, there are still unmet needs in patients with persistent disease activity, disease flares, decreased health-related quality of life, organ damage development, intolerance to standard treatment and comorbidities. It is encouraging that a plethora of therapeutic agents are currently under evaluation, although there are occasional clinical trial failures.

Key words: *Systemic lupus erythematosus; treatment; B cell; clinical trial*

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic heterogeneous multisystem autoimmune disease. Patients with SLE are typically treated with corticosteroids and immunosuppressants. Novel developments in the treatment of patients with SLE have been recently reviewed [1]. The U.S Food and Drug Administration (FDA) has approved belimumab and anifrolumab for the treatment of patients with SLE who are receiving standard therapy, and voclosporin and the intravenous form of belimumab for the treatment of lupus nephritis. Telitacicept has been also approved for the treatment of SLE in China. Rituximab, a B cell depletion treatment, may also be administered according to the European League Against Rheumatism (EULAR) and American College of Rheumatology (ACR) guidelines in refractory lupus nephritis despite the fact that the two large clinical trials failed to meet their primary endpoints and is also often administered off-label for other manifestations, based on the encouraging results of a plethora of studies [2,3]. Failure to achieve remission of lupus nephritis may lead to end-stage renal disease due to irreversible damage of the kidneys. Measurement of proteinuria using the urine protein to creatinine ratio (UPCR) is a tool to assess disease activity in patients with lupus nephritis. Improvement of proteinuria at 12 months of treatment is associated with a favorable long-term renal outcome. Despite advantages, a complete renal response is infeasible in more than 40% of patients with renal involvement. Thus, there is a need to introduce and evaluate additional or new regimens that are summarized in Table 1.

Division of Rheumatology, Department of Internal Medicine, University Hospital of Patras, Patras 26500, Greece

Received: 10 Apr 2022; Accepted: 31 May 2022

Targeting cells

B cells

B cells play a central role in the pathogenesis of SLE. B cells are able to produce autoantibodies after their differentiation into plasma cells, secrete cytokines, and present autoantigens to T cells as well. Thus, targeting B cells in the treatment of SLE seems a reasonable strategy.

Table 1. *Trials of agents demonstrating favorable results in the treatment of patients with SLE.*

Regimen	Phase of the study / Indication	Primary endpoint	Result	
Obinutuzumab	II / lupus nephritis	Complete renal response at week 52	35% (Obinutuzumab) vs 23% (placebo), p=0.115	
Obexelimab	II / SLE	Loss of improvement at day 225	42% (obexelimab) vs 28.6% (placebo), p=0,18	
Lupuzor	III / SLE	SRI response at week 52	52.5% (lupuzor) vs 44.65% (placebo), p=0.26	
Daratumumab	Case report / SLE and lupus nephritis			
Belimumab	FDA approved / lupus nephritis	Primary efficacy renal response at week 104	43% (belimumab) vs 32% (placebo), p=0.03	
Telitacicept	2b / SLE	SRI-4 response at week 48	71.0% (80mg), 68.3% (160mg), 75.8% (240mg) vs 33.9% (placebo), p<0.001, p<0.001, p<0.001, respectively	
Anifrolumab	FDA approved / SLE	BICLA response at week 52	47.8% (anifrolumab) vs 31.5% (placebo), p=0.001	
Voclosporin	FDA approved / lupus nephritis	Complete renal response at week 52	40.8% (voclosporin) vs 22.5% (placebo), p<0.0001	
Sirolimus		1/2 / SLE	Decreases of BILAG and SLEDAI scores at each visit (months 1-12)	BILAG: 28.4 (baseline) vs 17.4 (month 12), p<0.001 SLEDAI: 10.2 (baseline) vs 4.8 (month 12), p<0.001
		Retrospective / lupus nephritis		Proteinuria: 2.8±1.9 at baseline vs 0.1±0.1 at month 36
VIB7734		I / SLE, Sjogren's and CLE		Median change in CLASI-A from baseline to month 3: -5 (50mg), -9.5 (150mg), -5 (placebo)
BIIB059		II / SLE (part A)	Change of total joint count from baseline to week 24 CLASI-A at week 16	-15.0 (450mg) vs -11,6 (placebo), p=0.037
		CLE (part B)		-38.78 (50mg), -47.91 (150mg), -42.48 (450mg) vs -14.49 (placebo), p=0.015, p<0.001, p=0.001

Obinutuzumab is a type II humanized anti-CD20 monoclonal antibody (mAb) causing a greater B cell elimination than rituximab which is a type I anti-CD20 monoclonal antibody. Contrary to rituximab that failed to demonstrate a clinically significant difference compared to placebo in the primary endpoint of complete renal response, obinutuzumab was tested in patients with lupus nephritis with encouraging results. A total of more than 100 patients with Class III or Class IV lupus nephritis was randomized to receive obinutuzumab or placebo along with corticosteroids and mycophenolate mofetil (MMF) [4]. Complete renal response was achieved in 35% of the patients in the obinutuzumab group and in 23% of the patients in the placebo group (p=0.115) at week 52. This favorable response was sustained in 41% of the patients in the obinutuzumab group and in 23% of the patients in the placebo group through week 104 (p=0.026). Serious infections were observed in 8% in the obinutuzumab group and in 18% in the placebo group. It was also noticed that patients who had achieved sustained B cell depletion had a more favorable outcome of their renal disease at week 76, emphasizing the important effect of B cell elimination in the disease progress [5]. A phase III study aims to demonstrate that obinutuzumab and MMF without oral corticosteroids is non-inferior to treatment with MMF and oral corticosteroids in achieving the primary outcome of complete renal response at week 52 [6].

Obexelimab is a mAb against the CD19 molecule expressed on the surface of B cells, but it also binds to the Fcγ receptor IIb (FcγRIIB) the only inhibitory Fcγ receptor on the surface of B cells. Thus, obexelimab suppresses the activation of B cells without depleting them. In a phase II study, 104 patients were randomly assigned to receive obexelimab or placebo after achieving low disease activity by intramuscular (IM) steroids and after discontinuing previous immunosuppression [7]. Maintenance of improvement was observed through day 225 in 42% of patients in the obexelimab group and in 28.6% of patients in placebo group (p=0.18). However, patients in the obexelimab group had a significantly longer time to loss-of-improvement (median: 230 vs 131 days for patients in the placebo group, p=0.025).

T cells

T cells also participate in the pathogenesis of SLE. Lulizumab is a mAb against CD28, the T cell co-stimulatory molecule that is essential for T cell activation. In a phase II 24-week study, lulizumab was administered in a dose of 12.5 mg/week or at doses of 1.25, 5 mg, 12,5 mg every other week or placebo along with standard treatment in 349 patients with SLE [8]. Disease activity indices, such as the British Isles Lupus Assessment Group Based Composite Lupus Assessment (BICLA) response rate, the CLASI (Cutaneous Lupus Erythematosus Disease Area and Severity Index), and the SLEDAI (Systemic Lupus Erythematosus Disease Activity Index) did not show significant changes between groups during treatment.

Rigerimod or Lupuzor is a peptide, a fragment of the small nuclear ribonucleoprotein U1-70K. It may act as an immunomodulator by binding to major histocompatibility complex (MHC) class II and hence inhibiting T-cell reactivity, resulting in a partial restoration of the immune tolerance. In a phase III study rigerimod was introduced subcutaneously at a dose of 200 mg every 4 weeks for 48 weeks in combination to standard treatment [9]. A small and without statistical significance better response rate was noticed over placebo (52.5% vs 44.6%, p=0.26). Obviously, approaches targeting T cells seem less effective. Co-stimulation blockade has not been efficacious in the treatment of SLE, pointing perhaps to different pathway targets.

Plasma cells

Daratumumab that has been approved for the treatment of multiple myeloma, is an IgG1k mAb against CD38 causing depletion of plasma cells. Long-lived plasma cells are residents in niches in the bone marrow or in inflamed tissue and they do not respond to immunosuppressants, including B-cell-targeting treatments. Two patients with severe manifestations of SLE received daratumumab at a dose of 16 mg/kg of body weight once a week for 4 weeks followed by maintenance treatment with I.V. belimumab [10]. Daratumumab treatment resulted in remarkable clinical outcomes including improvement not only of severe manifestations such as lupus nephritis, autoimmune hemolytic anemia and autoimmune thrombocytopenia but also of less severe manifestations such as arthritis, skin rashes, pericarditis, cutaneous vasculitis, alopecia and mucosal ulcers. Favorable serologic responses were also observed. Importantly, previous therapeutic interventions with a variety of agents such as bortezomib, mycophenolate mofetil, and cyclophosphamide were ineffective. Despite the extremely small number of patients, data are encouraging supporting the further evaluation of daratumumab in adequately powered trials of patients with SLE. Of note, the clinical efficacy

of daratumumab was not associated exclusively to plasma cell depletion. Other circulating cells also express CD38 and their numbers were decreased after treatment with daratumumab. These include B cell subsets, plasmacytoid dendritic cells and an expanded CD38+ T cell subpopulation.

Plasmacytoid dendritic cells

Plasmacytoid dendritic cells have the ability to secrete massive amounts of type 1 interferons when activated contributing, thereby, to SLE pathogenesis. VIB7734 is a mAb that binds to ILT7, a surface molecule of pDCs, resulting in their elimination and also in the reduction of other cytokines such as IL-6 and TNF-α. A phase I, randomized, placebo-controlled trial evaluated VIB7734 in 3 cohorts [11]. Cohort 1 included 6 patients with SLE or Sjogren's syndrome with or without active disease. Cohorts 2 and 3 included patients with SLE or cutaneous lupus erythematosus (CLE) with a CLE Disease Area and Severity Index Activity Score (CLASI-A) ≥ 8. The median change of CLASI-A from baseline to month 3 was -5 in the 50mg, -9.5 in the 150mg group and -5 in the placebo group. Additionally, a ≥50% improvement in CLASI-A was observed in 56% of the patients treated with VIB7734 and in 29% of the patients in the placebo group at month 3. Treatment with VIB7734 was generally safe.

BIIB059 is a humanized IgG1 mAb that binds to the specific receptor of pDC BDCA2 (blood dendritic cell antigen 2) and suppresses the production of IFN-I. A 2-part phase II study assessed the effect of BIIB059 in patients with SLE (part A) and in patients with CLE (part B) [12]. The study achieved its primary endpoint which was the change in totally inflamed joints (tender and swollen joints) between baseline and week 24. Total active joint count significantly decreased in the BIIB059 450mg group [-15.0 vs -11.6 in the placebo group (p=0.037)]. A non-statistically significant increased CLASI-50 response was noticed in the BIIB059 group vs placebo. Adverse events were recorded in 67.9% in the placebo group and in 59.2% in the BIIB059 group. A further evaluation of part B illustrated a statistically significant change of CLASI-A score from baseline to week 16. Patients with severe SLE manifestations were not included in the study [13].

Treatments that affect B cell survival

Belimumab is a specific inhibitor of the soluble BLyS (B lymphocyte stimulator). The large belimumab's approval clinical trials had excluded patients with severe lupus nephritis. Of note, we had previously reported two patients in which lupus nephritis manifested shortly after the initiation of belimumab treatment [14]. Both these patients improved immediately after withdrawal of belimumab and before the initiation of standard therapy. Furthermore, a retrospective study showed that introducing belimumab into a standard treatment regimen of patients with SLE without renal involvement resulted in development of lupus nephritis with an increased frequency compared to patients with non-renal SLE who did not receive belimumab (hazard ratio, HR: 10.7, p=0.012) [15]. It was proposed that concomitant treatment with antimalarials was protective over this "nephritogenic" effect of belimumab ((HR: 0.2, p=0.046).

An international phase III, 104-week, randomized, double-blind, placebo-controlled trial of intravenous belimumab along with standard treatment formally addressed the question of its efficacy and safety in lupus nephritis [16]. A total of 448 patients were randomized to receive belimumab or placebo (1:1). The primary endpoint was primary efficacy renal response at week 104, an endpoint that was defined as a urinary protein to creatinine ratio (UPCR) ≤ 0.7, an estimated glomerular filtration rate (eGFR) that had not declined more than 20% below the levels before the flare of the disease or was >60ml/min/1.73 m^2, as well as no use of rescue treatment in cases of treatment failure. Primary efficacy renal response was noticed in 43% of the patients that were treated with belimumab along with standard therapy and in 32% of the patients that were treated with placebo in combination with standard treatment at week 104 (p=0.03). Regarding safety, no differences were observed between the two groups of patients. Although a significant number of patients with lupus nephritis was enrolled in each arm of the study, no subgroups of patients that could have a greater benefit from belimumab treatment were identified. Despite the fact that a better outcome was noticed in 11% more patients, the percentages of patients with renal response are not sufficient satisfying. The FDA has approved intravenous belimumab for the treatment of patients with lupus nephritis. Treatment with belimumab at the time that circulating BLyS peaks following rituximab treatment in order to sustain B cell depletion seems reasonable. However, a phase II study that examined the effect of induction therapy with rituximab followed by maintenance therapy with belimumab did not demonstrate a significant improvement of patients with lupus nephritis [17].

A randomized trial currently investigates the long-term efficacy of combination B cell targeting by initiation treatment with belimumab followed by rituximab in patients with lupus nephritis. The primary outcome is treatment failure rate at week 104 [18].

Bruton's tyrosine kinase (BTK) is an intracellular signaling molecule that plays an essential role in the activation, differentiation and survival of B cells. Fenebrutinib is a highly selective inhibitor of Bruton's tyrosine kinase (BTK). A phase II study that enrolled 260 SLE patients from 12 countries was conducted [19]. The SRI-4 response rates at week 48 were 51% in the fenebrutinib 150mg once daily group (p=0.37 vs placebo), 52% in the fenebrutinib 200mg twice daily group (p=0.34 vs placebo) and 44% in the placebo group. Although fenebrutinib illustrated an acceptable safety profile, it failed to improve disease activity.

Targeting other signals of B cell survival

Telitacicept (RC18) is a novel recombinant TACI-Fc (transmembrane activator and calcium modulator and cyclophilin ligand interactor) fusion protein that binds to soluble BLyS and APRIL (A proliferation inducing ligand) suppressing thereby their biological activities, that also affect the plasma cells. Therefore, telitacicept does not affect early and memory B cells. In a phase 2b study, patients with a Safety of Estrogen in Lupus Erythematosus National Assessment (SELENA)-SLEDAI score ≥ 8, consistent with active disease, received telitacicept at doses of 80mg, 160mg and 240mg or placebo along with standard treatment [20]. The primary endpoint was an SRI-4 at week 48. The SRI-4 was achieved in 71.0%, 68.3% and 75.8% of the patients who received the 80mg, 160mg and 240mg doses, respectively, at week 48 and in 33.9% of the patients in the placebo group. The proportion of patients achieving at least a 4-point reduction in their SELENA-SLEDAI score at week 48 was 75.8%, 77.8% and 79.0% in the telitacicept groups and 50.0% in the placebo group. Adverse events were observed in 90.3%, 92.1%, 93.5% and 82.3% of the patients in the 80mg, 160mg, 240mg telitacicept and placebo groups, respectively. Adverse events included mainly reactions at the injection site and infections of the upper respiratory tract. In March 2021, telitacicept was firstly approved in China for the treatment of patients with active SLE. Telitacicept is also evaluated in a phase III placebo-controlled study along with standard treatment. The primary outcome is SRI response rate at week 52 [21].

Targeting the IFN pathway

Anifrolumab is a fully human mAb that binds to the type I interferon receptor, blocking the activity of type I interferons such as interferon-α and interferon-β. A phase III, randomized, double-blind, placebo-controlled study included 362 patients with SLE [22]. They were randomized to receive anifrolumab (n=180) or placebo (n=182). A BICLA response was achieved in 47.8% of the patients in the anifrolumab group and in 31.5% of the patients in the placebo group at week 52. For patients with a high interferon gene signature, the percentages were 48.0% in the anifrolumab group and 30.7% in the placebo group. For patients with a low interferon gene signature, the percentages were almost similar to those with a high interferon gene signature (46.7% and 35.5%, respectively). Anifrolumab treatment also resulted in a reduction of glucocorticoid dosages and an improvement of skin involvement. Anifrolumab did not illustrate significant effects in arthritis or in annualized flare rates. Serious adverse events including pneumonia and deterioration of SLE were reported in 8.3% of the patients in the anifrolumab group and in 17% of the patients in the placebo group. Herpes zoster infection developed in 7.2% in the anifrolumab group and in 1.1% in the placebo group. Anifrolumab (Saphnelo) has been approved by the FDA for the treatment of adult patients with moderate to severe SLE who are receiving standard treatment. An ongoing phase III randomized, placebo-controlled trial currently evaluates the efficacy and safety of anifrolumab compared to placebo as added to standard treatment in patients with lupus nephritis. The primary outcome is complete renal response at week 52 [23].

Targeting specific intracellular pathways

Calcineurin inhibitors suppress the rapid ionized calcium infux that induces calcineurin activation in T cells following activation of BCR and TCR. Voclosporin is a novel cyclosporine analog, the most potent and least toxic among all known calcineurin inhibitors. A phase 3 study demonstrated that the addition of voclosporin to mycophenolate mofetil and low-dose corticosteroids was superior to standard treatment in patients with lupus nephritis. The AURORA study included 357 patients with active lupus nephritis [24]. Renal response was achieved in 40.8% of the patients in the voclosporin group and 22.5% of those in the control group and therefore the study met its primary endpoint. Patients receiving voclosporin had a 50% reduction in

the UPCR more rapidly than the control group. Serious adverse events, mainly infections were recorded in 20.8% of the patients in the voclosporin group and in 21.3% in the control group. Renal response at week 24, partial renal response at weeks 24 and 52, time to achieve UPCR ≤ 0.5, and time for 50% reduction of UPCR were the secondary endpoints and they all displayed a statistical significance in favor of voclosporin compared to standard treatment alone. There was no significant reduction of the eGFR at week 52 in the voclosporin group or increases of glucose, lipid levels or in blood pressure, which constitute common side effects of calcineurin inhibitors. A 2-year, controlled extension trial (AURORA 2) with a follow-up of 30 months included 90 patients in the voclosporin group and 78 patients in the control group [25]. According to these results, there were sustained meaningful reductions in proteinuria and no fluctuations of renal function through month 30. Voclosporin has been approved by the FDA as the first orally administered treatment for patients with lupus nephritis.

Targeting mTOR

Sirolimus is an immunosuppressive macrolide. It blocks the activation of T cells and B cells through mTOR (mammalian target of rapamycin) suppression, decreasing thereby their sensitivity to IL-2. In a prospective, open-label, single-arm clinical trial sirolimus was administered in 40 patients with SLE for 12 months [26]. Patients with severe or life-threatening manifestations of SLE, proteinuria (an UPCR more than 0.5) and hematological abnormalities had been excluded. Eleven patients discontinued the study due to lack of tolerance or lack of compliance. SLEDAI and BILAG scores were reduced in 16 out of 29 patients that completed treatment. Mean SLEDAI score was decreased from 10.2 at enrollment to 4.8 at 12 months after treatment ($p<0.001$). Mean BILAG score was decreased from 28.4 at enrollment to 17.4 at 12 months after treatment ($p<0.001$). The mean daily dose of prednisone was decreased from 23.7mg to 7.2mg ($p<0.001$) at 12 months after sirolimus initiation.

A retrospective study included 16 patients with class III and/or V or IV and/or V or pure class V lupus nephritis who received sirolimus [27]. Nine patients had intolerance to standard treatment (MMF and calcineurin inhibitors), and 7 patients had a history of cancer. Sirolimus was introduced as an induction treatment in 5 and as maintenance therapy in 11 patients. Proteinuria was diminished from 2.8±1.9 g/d at baseline to 0.1±0.1 g/d ($p=0.011$) at 36 months after treatment in the first group. A stable renal function was achieved in the second group. one patient experienced a renal flare and another one developed end-stage renal disease at 27 months after sirolimus treatment.

A meta-analysis was conducted to determine the overall efficacy of sirolimus in patients with SLE [28]. The overall reduction of SLEDAI and BILAG scores and that of corticosteroid dosages was 4.85, 1.98 and 13.17mg/d respectively in 111 patients with active disease. Remission was observed in 74% of the patients who received sirolimus for their active disease and maintenance of remission was achieved in 95.5% of the patients with lupus nephritis. Side effects were mild; only 9.3% of the patients discontinued treatment. It is therefore plausible that mTOR suppression may represent a promising novel therapeutic approach for patients with SLE.

Targeting the JAK-STAT pathway

The activation of the JAK-STAT pathway participates in the differentiation of pathogenic effector T cells and in the dysregulation of Treg cels. Baricitinib is an oral inhibitor of Janus kinase (JAK), blocking the subtypes JAK1 and JAK2. In a phase 2, double-blind, randomized, placebo-controlled, multicenter, 24-week study, 314 patients with active SLE involving skin or joints were randomized to receive baricitinib 4mg/d (n=104), baricitinib 2mg/d (n=105), or placebo (n=105) [29]. At week 24, reductions of SLEDAI scores were noticed in 67% of the patients in the baricitinib 4mg/d arm and in 58% of the patients in the baricitinib 2 mg/d arm. The higher dose of baricitinib (4mg/d) appeared to be more effective in the management of patients with SLE refractory standard treatment. Severe infections were recorded in 6% of the patients in the baricitinib 4 mg/d group, in 2% of the patients in the baricitinib 2 mg/d group and in 1% of the patients in the placebo group. Of note, deep vein thrombosis was observed in 1 patient receiving the 4 mg dosage regimen; this patient was positive for antiphospholipid antibodies.

In the phase III (SLE-BRAVE-I) study, the baricitinib 4mg regimen met the primary endpoint, illustrating a statistically significant reduction in disease activity as measured by the proportion of adult patients with active SLE who had an SRI-4 response at week 52 compared to placebo. However, the SLE-BRAVE-II study failed to meet the primary endpoint of SRI-4 response. In addition, important secondary endpoints were not met in either study. Based on top-line efficacy results from

the above phase-3 studies, the manufacturer decided to discontinue the phase-3 development program for baricitinib in patients with SLE [30]. Notably, safety that represented a major issue for JAK-inhibitors in rheumatoid arthritis patients with cardiovascular risk factors did not influence the manufacturer's decision.

Targeting cytokines interleukin 12 and 23

Ustekinumab is a mAb that binds to the p40 subunit of IL-12 and IL-23 preventing their binding to their receptors. A multicenter, randomized, double-blind, placebo-controlled study included 102 patients with active SLE. These patients were randomized to receive either ustekinumab or placebo along with standard treatment. SRI-4 response rates were significantly greater in the ustekinumab group (62%) compared to the placebo group (33%) at week 24 and were sustained through week 48. Ustekinumab was generally safe, since no opportunistic infections or deaths were recorded. The encouraging results of the phase II trial led investigators to design a phase III study. The manufacturer announced discontinuation of this study due to lack of efficacy resulting in the exclusion of ustekinumab from the therapeutic alternative approaches of SLE. The safety profile of ustekinumab was consistent with that of previous studies and did not impact the decision to discontinue the clinical trial.

Ongoing clinical trials

Apart from trials that were mentioned above, other ongoing studies are described below.

Treatments against T cells

Acazicolcept (ALPN-101) is a dual inhibitor of the CD28 and ICOS T cell co-stimulatory receptors that regulate the activation, proliferation and differentiation of T cells. It was created by engineering a single protein domain, or vIgD (Variant Ig Domain) based on a human inducible T cell co-stimulatory ligand (ICOSL) that is able to bind to CD28 and ICOS. A first-in-human study assessed the tolerability, safety, pharmacokinetics and pharmacodynamics of ALPN-101 in healthy adults [31]. According to the results, ALPN-101 seems to be well-tolerated with no clinically significant immunogenicity, evidence of cytokine release or severe side effects. A phase 2, randomized, blinded study aims to evaluate the safety and efficacy of ALPN-101 in patients with moderate to severe SLE [32].

Dapirolizumab pegol is an anti-CD40L pegylated Fab fragment that blocks co-stimulatory interactions between T cells and antigen presenting cells expressing CD40. A phase 2b study of dapirolizumab pegol in patients with SLE failed to meet the primary endpoint which was the dose-response at week 24, despite the fact that it was well-tolerated and showed improvements in disease activity [33]. However, investigators continue their research in order to determine the efficacy of dapirolizumab in a phase III study [34]. The primary outcome is BICLA response at week 48.

Itolizumab (EQ001) is a monoclonal antibody targeting the CD6 receptor on the surface of T cells. It blocks the binding of CD6 on ICAM (activated leukocyte cell adhesion molecule) ligand, inhibiting therefore immune responses mediated by T cells. CD6 and ALCAM positive cells were found to be increased in patients with lupus nephritis and to be associated with SLE activity [35]. Itolizumab improved renal disease in murine models, reduced the migration of T cells to inflamed tissues and also increased the levels of IL-10. Based on previous animal model data, the manufacturer was granted a U.S. FDA fast-track designation for itolizumab for the treatment of patients with lupus nephritis.

LY3471851 (NKTR-358) targets the IL-2 receptor complex and represents a novel Treg cell stimulator. It is designed to correct specifically this immune system dysregulation without affecting the entire immune system. The primary outcome of a phase II study is the percentage of patients that will achieve a ≥4-point reduction in SLEDAI-2K score at week 24 [36].

Abatacept is a fusion protein of the extracellular domain of CTLA4 and human IgG1-Fc, constructed to suppress B cell/T cell co-stimulation. Previous studies of abatacept failed to demonstrate benefit, even after withdrawal of background treatments. Nevertheless, a phase 2 study will evaluate the efficacy of abatacept in patients with SLE and the primary endpoint is BICLA response at 6 months [37].

Treatments against mainly (but not only) B cells

ALPN-303 inhibits B cell cytokines BLyS and APRIL, which play an important role in B cell survival, with higher than fivefold potency in vitro. In a mouse model of lupus, ALPN-303 treatment significantly decreased anti-dsDNA autoantibody levels and glomerulonephritis, whereas renal function remained stable and overall survival was improved [38]. Patients are currently enrolled in a phase 1 healthy volunteer clinical trial [39].

Iberdomide (CC220) is a cereblon modulator causing

potent degradation of the transcriptional factors Ikaros and Aiolos leading to suppressed B cell proliferation and cytokine secretion. A phase II, placebo-controlled study aims to evaluate the efficacy and safety of CC220 in patients with active SLE and the primary outcome is an SRI-4 at week 24 [40]. B cell and T cell collaboration plays a central role in the pathogenesis of SLE. Thus, AMG 570, an ICOSL and BAFF bispecific inhibitory molecule, has been employed in a phase 2b study [41]. The primary outcome is the percentage of patients achieving an SR-4 at week 52. Based on the same concept, VAY736 or lanalumab, a mAb that blocks the FAFF receptor and CFZ533 or iscalimab, a mAb that blocks CD40 pathway signaling are under investigation in a phase 2 study in patients with SLE and the primary outcome is an SRI-4 response at week 29 [42].

A phase Ib/IIa of orelabrutinib (ICP-022) aims to assess the safety, preliminary efficacy and tolerability in patients with mild to moderate SLE [43]. A phase 2 study aims to evaluate the safety and effectiveness of branebrutinib in patients with autoimmune diseases including SLE [44]. Elsubrutinib and upadacitinib treatments that are given alone or in combination are currently evaluated in patients with moderate to severe SLE [45]. The primary outcome of this phase 2 study is the achievement of SRI-4 and steroid dose ≤ 10mg prednisone equivalent once a day at week 24.

A 76-week, 3-part 1b/2 study aims to assess the pharmacological properties, safety and preliminary efficacy of tofacitinib in young adults with moderate to severe skin involvement due to lupus [46]. Brepocitinib (JAK1 and TYK2 inhibitor) is currently under evaluation in a dose-ranging phase 2 study in patients with SLE refractory to standard treatment [47]. The primary endpoint is SRI-4 at week 52. Deucravacitinib (TYK2 inhibitor) is under investigation in a phase 2 study in patients with lupus nephritis [48].

Secukinumab is a human IgG1 monoclonal antibody that selectively binds to interleukin-17A (IL-17A). A phase III study evaluates the efficacy and safety of secukinumab in combination with standard treatment in patients with active lupus nephritis. The primary outcome is the proportion of patients achieving complete renal response at week 52 [49].

Other potential treatments

Low-dose IL-2 might also be effective in patients with SLE. A randomized, placebo-controlled study showed that the SRI-4 response rate was 65.2% of the patients in the IL-2 group and 36.7% in the placebo group (p=0.027) at week 24 [50]. The primary endpoint which was the SRI-4 response at week 12 was not met. Regarding lupus nephritis, complete renal response was achieved in 53.85% of the patients in the IL-2 group compared to 16.67% in the placebo group (p=0.036). A multicenter, double-blind, placebo-controlled trial will establish which of the three different dosages of IL-2 would be more efficacious and safer for patients with SLE [51].

Lenabasum is a synthetic endocannabinoid receptor type 2 agonist that activates innate immunity without immunosuppression. A phase 2 study evaluates the efficacy and safety of lenabasum in SLE patients with active joint disease and at least moderate pain [52]. It has been found that a single nucleotide polymorphism in the gene for the N2A subunit of the N-methyl-D-aspartate (NMDA) receptor, GRIN2A, encodes augmented NMDA receptor activity. Memantine is an NMDA receptor antagonist and is currently evaluated in a phase 2 study in patients with cognitive dysfunction [53]. A phase 1b/2 study aims to evaluate KZR-616 (a selective immunoproteasome inhibitor) in SLE patients with and without renal involvement [54].

Cenerimod is a selective agonist for the G-protein-coupled sphingosine-1-phosphate receptor 1 (S1P receptor 1 or S1P1), also known as endothelial differentiation gene 1 (EDG1). It seems to be a potent immunomodulator due to its effects in the number of circulating and infiltrating T- and B-cells. In a phase II study cenerimod was introduced at different doses in patients with SLE [55]. T- and B-cells were measured by flow cytometry before and at 12 weeks after treatment. There was a reduction of CD4+T cells (95%) and CD19+B cells (90%) and also a reduction of antibody-secreting cells (85%). The safety profile of this agent is unknown. The purpose of a phase 2 study is to evaluate the efficacy and safety of 4 doses of cenerimod in patients with SLE [56]. The primary outcome is change of the modified SLEDAI from baseline to month 6.

Other potential therapeutic approaches such as Epstein Barr Virus-specific cytotoxic T lymphocytes [57], mesenchymal stem cells [58,59] and curcumin are also currently under investigation in patients with SLE [60].

DISCUSSION

This review demonstrates the continuous efforts in order to achieve a sufficient control of the manifestations of SLE. The trials that have been conducted or are currently under way, include a variety of regimens due

to the plethora of the disturbances implicated in SLE pathogenesis.

Lupus nephritis is an aspect of the disease often difficult to treat. Fortunately, two drugs, the orally administered voclosporin and the intravenous form of belimumab, have been recently approved by the FDA for the treatment of patients with lupus nephritis on top of standard of care. Regarding voclosporin, besides its efficacy persisting at 30 months, no drug interaction with MMF has been observed, while it is also associated with a more favorable effect on glucose and lipid levels than other calcineurin inhibitors. On the other hand, belimumab prevents time-to-organ damage progression, while it is not associated with arterial hypertension. A report suggests daratumumab that targets plasma cells as well as other immune cells, as an alternative therapeutic approach for patients with SLE. However, studies with greater numbers of patients are necessary to determine the efficacy and safety of daratumumab in patients with SLE. A pilot study suggests that the mTOR inhibitor sirolimus could also be a generally safe and well tolerated alternative therapeutic approach in the management of lupus nephritis in patients who are intolerant to standard treatment or in cases of a history of malignancy. Treatment options for other aspects of the disease such as neuropsychiatric involvement are quite limited. A common symptom that dramatically decreases the quality of patients' lives is fatigue and cannot be managed sufficiently so far.

Sometimes a combination of treatments might be necessary to be introduced, since lupus is a multifactorial disease. Generalized immunosuppression should be minimized with the administration of novel agents, because infections that are potentially life threatening are always an important issue.

CONCLUSIONS

SLE is a multifactorial disease often difficult to manage. Occasionally, combination therapy is mandatory to control disease activity. Due to the extreme heterogeneity of the disease, personalized approaches might also be important. Apart from the efficacy of a treatment, the safety profile is another important issue.

Acknowledgments: *I wish to thank Professor Stamatis-Nick Liossis for guidance and mentorship on lupus related autoimmunity throughout the years.*

Conflict of interest disclosure: *None to declare*

Declaration of funding sources: *None to declare*

Author Contributions: *C. Staveri, study conception and design, analysis and interpretation of data, drafting of manuscript.*

REFERENCES

1. Liossis SN, Staveri C. What's New in the Treatment of Systemic Lupus Erythematosus. Front Med (Lausanne). 2021;8:655100.
2. Staveri C, Liossis SN. Rituximab for SLE refractory to conventional treatment: results of a cohort evaluating efficacy and long-term outcome. Clin Exp Rheumatol. 2016;34(4): S5-5.
3. Liossis SC, Staveri C. B Cell-Based Treatments in SLE: Past Experience and Current Directions. Curr Rheumatol Rep. 2017;19(12):78.
4. Furie RA, Aroca G, Cascino MD, Garg JP, Rovin BH, Alvarez A, et al. B-cell depletion with obinutuzumab for the treatment of proliferative lupus nephritis: a randomised, double-blind, placebo-controlled trial. Ann Rheum Dis. 2022;81(1):100-7.
5. Furie R, Cascino MD, Garg JP, Aroca G, Alvarez A, Fragoso-Loyo H, et al. B cell depletion and response in a randomized, controlled trial of obinutuzumab for proliferative lupus nephritis. 035 Oral Presentations. Lupus Sci Med. 2020;7(Suppl 1):A27.
6. Clinical trials.gov. Induction Therapy for Lupus Nephritis With no Added Oral Steroids: A Trial Comparing Oral Corticosteroids Plus Mycophenolate Mofetil (MMF) Versus Obinutuzumab and MMF (OBILUP). https://clinicaltrials.gov/ct2/show/NCT04702256
7. Merrill JT, June J, Koumpouras F, Machua W, Khan MF, Askanase A, et al. Top-line results of a phase 2, double-blind, randomized, placebo-controlled study of a reversible B cell inhibitor, XmAb®5871, in systemic lupus erythematous (SLE). Arthritis Rheumatol. 2018;70(suppl 10):L14.
8. Merrill JT, Shevell DE, Duchesne D, Nowak M, Kundu S, Girgis IG, et al. An Anti-CD28 Domain Antibody, Lulizumab, in Systemic Lupus Erythematosus: Results of a Phase II Study. Arthritis Rheumatol. 2018;70(suppl 10).
9. ImmuPharma. Top line results of LupuzorTM Pivotal Phase III Trial (2018). https://www.immupharma.co.uk/top-line-results-lupuzor-pivotal-phase-iii-trial/
10. Ostendorf L , Burns M , Durek P, Heinz GA , Heinrich F , Garantziotis P , et al. Targeting CD38 with Daratumumab in Refractory Systemic Lupus Erythematosus. N Engl J Med. 2020;383(12):1149-55.
11. Werth V, Karnell J, Rees W, Mittereder N, Yan L, Wu Y, et al. Targeting Plasmacytoid Dendritic Cells Improves Cutaneous Lupus Erythematosus Skin Lesions and Reduces Type I Interferon Levels: Results of a Phase 1 Study of VIB7734. 2020. Arthritis Rheumatol. 2020;72 (suppl 10):L10.
12. Furie R, van Vollenhoven R, Kalunian K, Navarra S, Romero-Díaz J, Werth V, et al. Efficacy and Safety Results from a Phase 2, Randomized, Double-Blind Trial of BIIB059, an Anti-Blood Dendritic Cell Antigen 2 Antibody, in SLE. Arthritis Rheu-

matol. 2020; 72 (suppl 10).
13. Werth V, Furie R, Romero-Díaz J, Navarra S, Kalunian K, van Vollenhoven R, et al. BIIB059, a Humanized Monoclonal Antibody Targeting Blood Dendritic Cell Antigen 2 on Plasmacytoid Dendritic Cells, Shows Dose-Related Efficacy in a Phase 2 Study in Participants with Active Cutaneous Lupus Erythematosus. Arthritis Rheumatol. 2020; 72 (suppl 10).
14. Staveri C, Karokis D, Liossis SC. New onset of lupus nephritis in two patients with SLE shortly after initiation of treatment with belimumab. Semin Arthritis Rheum. 2017;46(6):788-90.
15. Parodis I, Vital EM, Hassan SU, Jönsen A, Bengtsson AA, Eriksson P, et al. De novo lupus nephritis during treatment with belimumab. Rheumatology (Oxford). 2021;60(9):4348-54.
16. Furie R , Rovin BH , Houssiau F, Malvar A, Teng YKO, Contreras G, et al. Two-Year, Randomized, Controlled Trial of Belimumab in Lupus Nephritis. N Engl J Med. 2020;383(12):1117-28.
17. Atisha-Fregoso Y , Malkiel S , Harris KM , Byron M , Ding L , Kanaparthi S, et al . Phase II Randomized Trial of Rituximab Plus Cyclophosphamide Followed by Belimumab for the Treatment of Lupus Nephritis. Arthritis Rheumatol. 2021;73(1): 121-31.
18. Clinical trials.gov. Synergetic B-cell Immunomodulation in SLE - 2nd Study. (SynBioSe-2) https://clinicaltrials.gov/ct2/show/NCT03747159?term=03747159&draw=2&rank=1
19. ClinicalTrials.gov. A study of the safety and efficacy of GDC-0853 in participants with moderate to severe active systemic lupus erythematosus. https://clinicaltrials.gov/ct2/show/NCT02908100.
20. Wu D, Li J, Xu D, Wang W, Li L, Fang J, et al. A Human Recombinant Fusion Protein Targeting B Lymphocyte Stimulator (BLyS) and a Proliferation-Inducing Ligand (APRIL), Telitacicept (RC18), in Systemic Lupus Erythematosus (SLE): Results of a Phase 2b Study. Arthritis Rheumatol. 2019; 71 (suppl 10).
21. Clinical.trials.gov. A Phase III, Placebo-Controlled, Multi-Center, Randomized, Double-Blind, Dose-exploring Trial of RC18,a Recombinant Human B Lymphocyte Stimulating Factor Receptor-Antibody Fusion Protein in Subjects With Systemic Lupus Erythematosus (SLE). https://clinicaltrials.gov/ct2/show/NCT04082416
22. Morand EF, Furie R, Tanaka Y, Bruce IN, Askanase AD, Richez C, et al. TULIP-2 Trial Investigators. Trial of Anifrolumab in Active Systemic Lupus Erythematosus. N Engl J Med. 2020;382(3):211-21.
23. Clinical trials.gov. Phase 3 Study of Anifrolumab in Adult Patients With Active Proliferative Lupus Nephritis (IRIS). https://clinicaltrials.gov/ct2/show/NCT05138133
24. Rovin BH, Teng YKO, Ginzler EM, Arriens C, Caster DJ, Romero-Diaz J, et al. Efficacy and safety of voclosporin versus placebo for lupus nephritis (AURORA 1): a double-blind, randomised, multicentre, placebo-controlled, phase 3 trial. Lancet. 2021;397(10289):2070-80.
25. Saxena A, Mela C, Coeshall A. Voclosporin for Lupus Nephritis: Interim Analysis of the AURORA 2 Extension Study [abstract]. Arthritis Rheumatol. 2021; 73 (suppl 10). https://acrabstracts.org/abstract/voclosporin-for-lupus-nephritis-interim-analysis-of-the-aurora-2-extension-study/. Accessed April 4, 2022.
26. Lai ZW, Kelly R, Winans T, Marchena I, Shadakshari A, Yu J, et al. Sirolimus in patients with clinically active systemic lupus erythematosus resistant to, or intolerant of, conventional medications: a single-arm, open-label, phase 1/2 trial. Lancet. 2018;391(10126):1186-96.
27. Yap DYH, Tang C , Chan GCW , Kwan LPY , Ma MKM , Mok MMY , Chan TM. Longterm Data on Sirolimus Treatment in Patients with Lupus Nephritis. J Rheumatol. 2018;45(12):1663-70.
28. Ji L , Xie W, Zhang Z. Efficacy and safety of sirolimus in patients with systemic lupus erythematosus: A systematic review and meta-analysis. Semin Arthritis Rheum. 2020;50(5):1073-80.
29. Wallace DJ, Furie RA, Tanaka Y, Kalunian KC, Mosca M, Petri MA, et al. Baricitinib for systemic lupus erythematosus: double-blind, randomized, placebo-controlled, phase 2 trial. Lancet. 2018.392(10143):222-31.
30. https://www.prnewswire.com/news-releases/updates-on-olumiant-baricitinib-phase-3-lupus-program-and-fda-review-for-atopic-dermatitis-301470359.html
31. Yang J, Lickliter JD, Hillson JL, Means GD, Sanderson RJ, Carley K, et al. First-in-human study of the safety, tolerability, pharmacokinetics, and pharmacodynamics of ALPN-101, a dual CD28/ICOS antagonist, in healthy adult subjects. Clin Transl Sci. 2021;14(4):1314-26.
32. Clinical trials.gov. ALPN-101 in Systemic Lupus Erythematosus https://www.clinicaltrials.gov/ct2/show/NCT04835441
33. Furie RA, Bruce IN, Dörner T, Leon MG, Leszczyński P, Urowitz M, et al. Phase 2, randomized, placebo-controlled trial of dapirolizumab pegol in patients with moderate-to-severe active systemic lupus erythematosus. Rheumatology (Oxford). 2021;60(11):5397-407.
34. ClinicalTrials.gov. A study to evaluate the efficacy and safety of dapirolizumab pegol in study participants with moderately to severely active systemic lupus erythematosus (PHOENYCS GO). https://clinicaltrials.gov/ct2/show/NCT04294667.
35. Lupus News today. Itolizumab granted FDA fast track designation as potential treatment for lupus nephritis. https://lupusnewstoday.com/2019/12/18/itolizumab-granted-fda-fast-track-designation-as-lupus-nephritis-treatent/. [Accessed December 18,2019].
36. Clinical trials.gov. A Randomized, Double-Blind, Placebo-Controlled, Phase 2 Study of LY3471851 (NKTR-358) in Adults With Systemic Lupus Erythematosus. https://clinicaltrials.gov/ct2/show/NCT04433585
37. Clinical trials.gov. Clarification of Abatacept Effects in SLE With Integrated Biologic and Clinical Approaches (ABC). https://clinicaltrials.gov/ct2/show/NCT02270957.
38. Dillon S, Evans L, Lewis K, Yang J, Rixon M, Kuijper J, et al. ALPN-303, an Enhanced, Potent Dual BAFF/APRIL Antagonist Engineered by Directed Evolution for the Treatment of Systemic Lupus Erythematosus (SLE) and Other B Cell-Related Diseases. Arthritis Rheumatol. 2021;73 (suppl 10).
39. Clinical trials.gov. A Study of ALPN-303 in Adult Healthy Vol-

unteers. https://clinicaltrials.gov/ct2/show/NCT05034484
40. Clinical trials.gov. A Phase 2, Multicenter, Randomized, Double-Blind, Placebo-Controlled Study to Evaluate the Efficacy and Safety of CC-220 in Subjects With Active Systemic Lupus Erythematosus. https://clinicaltrials.gov/ct2/show/NCT03161483
41. Clinical trials.gov. A Phase 2b Dose Ranging Study to Evaluate the Efficacy and Safety of Rozibafusp Alfa (AMG 570) in Subjects With Active Systemic Lupus Erythematosus (SLE) With Inadequate Response to Standard of Care (SOC) Therapy. https://clinicaltrials.gov/ct2/show/NCT04058028
42. Clinical trials.gov. A Placebo-controlled, Patient and Investigator Blinded, Randomized Parallel Cohort Study to Assess Pharmacodynamics, Pharmacokinetics, Safety, Tolerability and Preliminary Clinical Efficacy of VAY736 and CFZ533 in Patients With Systemic Lupus Erythematosus (SLE). https://clinicaltrials.gov/ct2/show/NCT03656562.
43. Clinical trials.gov. A Phase Ib/IIa Double-blind, Randomized, Placebo-controlled Study to Evaluate the Safety, Tolerance, Pharmacokinetics/ Pharmacodynamics(PK/PD) of ICP-022 in Patients With Mild and Moderate Systemic Lupus Erythematosus. https://clinicaltrials.gov/ct2/show/NCT04305197.
44. Clinical trials.gov. A Randomized, Placebo-Controlled, Double-Blind, Multicenter Study to Assess the Efficacy and Safety of Branebrutinib Treatment in Subjects With Active Systemic Lupus Erythematosus or Primary Sjögren's Syndrome, or Branebrutinib Treatment Followed by Open-label Abatacept Treatment in Subjects With Active Rheumatoid Arthritis. https://clinicaltrials.gov/ct2/show/NCT04186871.
45. Clinical trials.gov. A Phase 2 Study to Investigate the Safety and Efficacy of Elsubrutinib and Upadacitinib Given Alone or in Combination (ABBV-599 Combination) in Subjects With Moderately to Severely Active Systemic Lupus Erythematosus. https://clinicaltrials.gov/ct2/show/NCT03978520.
46. Clinical trials.gov. A 3-part Open-label Study Assessing Safety, Tolerability, Pharmacokinetic and -Dynamic Profiles, and Efficacy of Tofacitinib in Young Adults From Age 18 to 45 With Moderate to Severe Skin Involvement Due to Lupus. https://clinicaltrials.gov/ct2/show/NCT03288324.
47. Clinical trials.gov. A Phase 2b, Double-Blind, Randomized, Placebo-Controlled, Multicenter, Dose-Ranging Study to Evaluate the Efficacy and Safety Profile of PF-06700841 in Participants With Active Systemic Lupus Erythematosus (SLE). https://clinicaltrials.gov/ct2/show/NCT03845517.
48. Clinical trials.gov. A Multi-Center Study to Characterize the Long-Term Safety and Efficacy of BMS-986165 in Subjects With Systemic Lupus Erythematosus. https://clinicaltrials.gov/ct2/show/NCT03920267.
49. Clinical trials.gov. A two-year, phase III randomized, double-blind, parallel-group, placebo-controlled trial to evaluate the safety, efficacy, and tolerability of 300mg s.c. secukinumab versus placebo, in combination with SOC therapy, in patients with active lupus nephritis. clinicaltrials.gov/ct2/show/NCT04181762.
50. He J, Zhang R, Shao M, Zhao X, Miao M, Chen J, et al. Efficacy and safety of low-dose IL-2 in the treatment of systemic lupus erythematosus: a randomised, double-blind, placebo-controlled trial. Ann Rheum Dis. 2020;79(1):141-49.
51. Clinical trials.gov. Efficacy and Safety of Low-dose IL-2 in Patients With SLE: a Multicenter, Randomised, Placebo-controlled Trial https://clinicaltrials.gov/ct2/show/NCT04077684
52. Clinical trials.gov. A Phase 2, Double-blind, Randomized, Placebo-controlled Multicenter Study to Evaluate Efficacy, Safety, and Tolerability of JBT-101 in Systemic Lupus Erythematosus (ALE09). https://clinicaltrials.gov/ct2/show/NCT03093402.
53. Clinical trials.gov. A Randomized Placebo-controlled, Double Blind Phase 2 Clinical Trial of Memantine for the Treatment of Cognitive Impairment in Systemic Lupus Erythematosus. https://clinicaltrials.gov/ct2/show/NCT03527472.
54. Clinical trials.gov. A Study of KZR-616 in Patients With SLE With and Without Lupus Nephritis (MISSION). https://clinicaltrials.gov/ct2/show/NCT03393013
55. Strasser D, Sippel V, Grieder U, Kieninger-Graefitsch A, Pierlot G, Farine H, et al. Cenerimod, a Potent, Selective and Orally Active Sphingosine 1-phosphate Receptor 1 Modulator, Reduced Blood Antibody-secreting Cells in Patients with SLE. Arthritis Rheumatol. 2019;71(Suppl 10).
56. Clinical trials.gov. Efficacy and Safety of Four Doses of Cenerimod Compared to Placebo in Adult Subjects With Active Systemic Lupus Erythematosus. https://clinicaltrials.gov/ct2/show/NCT03742037?term=cenerimod&cond=lupus&draw=2&rank=1
57. Clinical trials.gov. Restauration of EBV Control in SLE Phase 1-2 Trial Evaluating Adoptive Transfer of Autologous EBV-Specific Cytotoxic T Lymphocytes in SLE Treatment. https://clinicaltrials.gov/ct2/show/NCT02677688.
58. Clinical trials.gov. A Phase II Controlled Trial of Allogeneic Mesenchymal Stem Cells for the Treatment of Refractory Lupus. https://clinicaltrials.gov/ct2/show/NCT02633163.
59. Clinical trials.gov. Dose-response and Efficacy of Umbilical Cord-derived Mesenchymal Stromal Cells in Renal Systemic Lupus Erythematosus. https://clinicaltrials.gov/ct2/show/NCT03917797.
60. Clinical trials.gov. Effect of Curcumin on Systemic Lupus Erythematosus. https://clinicaltrials.gov/ct2/show/NCT03953261.

Corresponding author:

Chrysanthi Staveri
Patras University Hospital, Department of Internal Medicine, Division of Rheumatology, Patras 26500, Greece
Tel.: +30 2613 603 693, Fax: +30 2610 993 982
E-mail: xrsta@upatras.gr

Achaiki Iatriki