Emerging immunolological strategies: recent advances and future directions

Hongyun Zhao², Fan Luo³, Jinhui Xue², Su Li², Rui-Hua Xu (✉)¹

¹Department of Medical Oncology, State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, Sun Yat-sen University Cancer Center, Guangzhou 510060, China; ²Department of Clinical Research, State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, Sun Yat-sen University Cancer Center, Guangzhou 510060, China; ³Department of Experimental Research, State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, Sun Yat-sen University Cancer Center, Guangzhou 510060, China

© The Author(s) 2021. This article is published with open access at link.springer.com and journal.hep.com.cn

Abstract Immunotherapy plays a compelling role in cancer treatment and has already made remarkable progress. However, many patients receiving immune checkpoint inhibitors fail to achieve clinical benefits, and the response rates vary among tumor types. New approaches that promote anti-tumor immunity have recently been developed, such as small molecules, bispecific antibodies, chimeric antigen receptor T cell products, and cancer vaccines. Small molecule drugs include agonists and inhibitors that can reach the intracellular or extracellular targets of immune cells participating in innate or adaptive immune pathways. Bispecific antibodies, which bind two different antigens or one antigen with two different epitopes, are of great interest. Chimeric antigen receptor T cell products and cancer vaccines have also been investigated. This review explores the recent progress and challenges of different forms of immunotherapy agents and provides an insight into future immunotherapeutic strategies.

Keywords cancer immunotherapy; bispecific antibodies; small molecules; chimeric antigen receptor T therapy; cancer vaccines

Introduction

Immunotherapy has brought tumor therapy into a new era. From surgery, to radiotherapy, chemotherapy, and targeted therapy, immuno-oncology therapy is almost within reach. However, many obstacles remain for this treatment. Although immune checkpoint inhibitors (ICIs) are now widely studied and have shown promising clinical data, many patients receiving ICIs fail to achieve clinical benefits, show varying response rates among different tumor types [1,2], and suffer from risk of immune-related adverse events (irAEs) [3].

New approaches that promote anti-tumor immunity have recently been developed, such as small molecules, bispecific antibodies (bsAbs), chimeric antigen receptor (CAR) T cell products, and even cancer vaccines. These new drugs can be used alone or in conjunction with existing biological antibodies and traditional therapies (radiotherapy or chemotherapy) to affect various members of the immune system and microenvironment, promote antitumor effectiveness, and benefit many patients.

This review explores the mechanisms and recent advances of small molecule drugs, bsAbs, cancer vaccines, and CAR T cell therapy. Challenges and future directions of these novel immunotherapy strategies are also discussed.

Small molecules in immunotherapy

An overview

With deepened understanding of innate immunity and tumor microenvironment (TME), many small molecules and their importance in cancer immunity have been discovered. Small molecule drugs include agonists and inhibitors that can reach the intracellular or extracellular targets of immune cells participating in specific immune pathways, enhancing anti-tumor immunity, or reducing
immune suppression. These substances also have potential complementary or synergistic effects with existing immunotherapy. Compared with therapeutic antibodies, small molecule drugs are more permeable to tissues and the TME, and can cross the blood–brain barrier and other physiologic barriers, thus providing new options for the treatment of brain tumors and brain metastases. By adjusting the pharmacokinetic and pharmacodynamic parameters, small molecule drugs may provide the best bioavailability and avoid some of the irAEs associated with long-lasting antibody therapies. These medications also have relatively low production costs and are usually taken orally which enables easy administration.

Although a growing number of small molecules have entered early phase clinical trials, many challenges remain to be solved. Specific issues relate to understanding their mechanisms of action in the immune system and the theoretical basis for further clinical applications, as well as, the need for more safety and efficacy evaluations.

**Mechanisms of small molecule drugs**

Over the past decade, more than 50 small molecule drugs have been produced as single agents or in combination with monoclonal antibodies for tumor immunotherapy [4], and over 100 clinical trials are currently underway (Table 1 and Fig. S1). Small molecule agonists and inhibitors target specific pathways participating in innate or adaptive immunity through different mechanisms (Fig. 1). Understanding the mechanism of small molecule drugs and their current clinical research progress will aid in exploring their role in immunotherapy.

| Small molecule | Target | Clinical studies | Phase   | Cancer type                                      |
|----------------|--------|------------------|---------|-------------------------------------------------|
| CA-170         | PD-L1/VISTA | NCT02812875     | Phase 1 | Advanced solid tumors or lymphomas              |
| Imiquimod      | TLR7   | Approved         |         | Ovarian cancer                                  |
| Motolimod      | TLR78  | NCT02431559      | Phase 1/2| Ovarian cancer                                  |
|                |        | NCT03906526      | Phase 1 | Head and neck cancer                            |
|                |        | NCT04272333      | Early phase 1 | Head and neck squamous cell carcinoma           |
|                |        | NCT02650635      | Phase 1 | Metastatic, persistent, recurrent, or progressive solid tumors |
|                |        | NCT02124850      | Phase 1 | Head and neck squamous cell carcinoma           |
| Resiquimod     | TLR7/8 | NCT00821652      | Phase 1 | Tumors                                          |
|                |        | NCT00948961      | Phase 1/2| Advanced malignancies                           |
|                |        | NCT00960752      | Phase 2 | Melanoma                                        |
|                |        | NCT01204684      | Phase 2 | Brain tumors                                    |
|                |        | NCT01808950      | Phase 1/2 | Nodular basal cell carcinoma                    |
|                |        | NCT00470379      | Phase 1 | Melanoma (skin)                                 |
|                |        | NCT01748747      | Phase 1 | Melanoma                                        |
|                |        | NCT02126579      | Phase 1/2| Melanoma                                        |
|                |        | NCT01676831      | Phase 1/2 | Cutaneous T cell lymphoma                       |
| VTX-2337       | TLR8   | NCT01666444      | Phase 1/2 | Epithelial ovarian cancer, fallopian tube cancer, primary peritoneal cancer |
|                |        | NCT01334177      | Phase 1 | Locally advanced, recurrent, or metastatic squamous cell cancer of head and neck |
|                |        | NCT03906526      | Phase 1 | Head and neck cancer                            |
|                |        | NCT01836029      | Phase 2 | Head and neck squamous cell carcinoma           |
|                |        | NCT02124850      | Phase 1 | Head and neck squamous cell carcinoma           |
| Epacadostat    | IDO1   | NCT03322540      | Phase 2 | Metastatic non-small cell lung cancer           |
|                |        | NCT03348904      | Phase 3 | Non-small cell lung cancer                      |
|                |        | NCT03322566      | Phase 2 | Metastatic non-small cell lung cancer           |
|                |        | NCT02959437      | Phase 1/2 | Advanced solid tumors                          |
|                |        | NCT03085914      | Phase 1/2 | Advanced or metastatic solid tumors            |
|                |        | NCT02318277      | Phase 1/2 | Advanced solid tumors                          |
|                |        | NCT03347123      | Phase 1/2 | Advanced or metastatic malignancies            |
|                |        | NCT03006302      | Phase 2 | Metastatic pancreas cancer                      |
|                |        | NCT03361865      | Phase 3 | Urothelial carcinoma                            |
|                |        | NCT02327078      | Phase 1/2 | B cell malignancies, colorectal cancer, head and neck cancer, lung cancer, lymphoma, melanoma, ovarian cancer, glioblastoma |
| Small molecule | Target | Clinical studies | Phase | Cancer type |
|----------------|--------|-----------------|-------|-------------|
| Navoximod (GDC-0919) | IDO1 | NCT02048709 | Phase 1 | Advanced solid tumors |
| BMS-986205 | IDO1 | NCT03519256 | Phase 2 | Bladder cancer |
| PF-06840003 | IDO1 | NCT02764151 | Phase 1 | Malignant gliomas |
| CB-1158 (INCB001158) | ARG | NCT03910530 | Phase 1 | Advanced solid tumors |
| AT-38 | ARG | NCT03910530 | Phase 1 | Advanced solid tumors |
| CB-839 | Glutaminase 1 | NCT03263439 | Phase 1 | Non-small cell lung cancer |
| CPI-444 (V81444; ciforadenant) | A2A receptor | NCT02655822 | Phase 1 | Renal cell cancer, metastatic castration resistant prostate cancer |
| Preladenant | A2A receptor | NCT03099161 | Phase 1 | Advanced cancers |
| PBF 509 | A2A receptor | NCT04680107 | Phase 1 | Non-small cell lung cancer |
| AZD4635 | A2A receptor | NCT04089553 | Phase 2 | Prostate cancer |
| ADU-S100 | STING | NCT03010176 | Phase 1 | Solid tumors, lymphoma |
| MK1454 | STING | NCT03172936 | Phase 1 | Solid tumors and lymphomas |
| Turalio (pexidartinib) (PLX3397) | CSF1R | NCT02777710 | Phase 1 | Metastatic/advanced pancreatic or colorectal cancers |
| | | NCT02734433 | Phase 1 | Advanced solid tumors |
| | | NCT01525602 | Phase 1 | Advanced solid tumors |
| | | NCT02452424 | Phase 1 | Advanced melanoma and other solid tumors |
| | | NCT01349036 | Phase 2 | Recurrent glioblastoma |
| | | NCT02975700 | Not applicable | Melanoma |
Targeting immune checkpoints

Programmed death protein 1 (PD-1) or programmed death protein-ligand 1 (PD-L1) antibodies have a long-half life and only act on extracellular PD-1/PD-L1, that is, they cannot penetrate the tissue barrier. Therefore, the

| Small molecule | Target               | Clinical studies | Phase      | Cancer type                                                                 |
|----------------|----------------------|------------------|------------|----------------------------------------------------------------------------|
| LYC-55716      | RORγt                | NCT02929862      | Phase 1/2  | Advanced or metastatic cancer                                               |
|                |                      | NCT03396497      | Phase 1    | Non-small cell lung cancer                                                  |
| TNO155         | SHP2                 | NCT04000529      | Phase 1    | Non-small cell lung carcinoma, head and neck squamous cell carcinoma, esophageal SCC, gastrointestinal stromal tumors, colorectal cancer |
|                |                      | NCT03114319      | Phase 1    | Advanced solid tumors                                                       |
| RMC-4630       | SHP2 (SAR442720)     | NCT03989115      | Phase 1/2  | Solid tumor                                                                 |
|                |                      | NCT03634982      | Phase 1    | Relapsed/refractory solid tumors                                            |
|                |                      | NCT04418661      | Phase 1    | Metastatic neoplasm                                                         |
| JAB-3068       | SHP2                 | NCT03518554      | Phase 1    | Advanced solid tumors                                                       |
| JAB-3312       | SHP2                 | NCT04121286      | Phase 1    | Advanced solid tumors                                                       |
| Idelalisib     | PI3K-δ               | Approved         |            |                                                                            |
| IPI-549        | PI3K-γ               | NCT03961698      | Phase 2    | Breast cancer, renal cell carcinoma                                          |
|                |                      | NCT03719326      | Phase 1    | Triple-negative breast cancer, ovarian cancer                                |
|                |                      | NCT02637531      | Phase 1    | Advanced solid tumors                                                       |
|                |                      | NCT03980041      | Phase 2    | Advanced urothelial carcinoma                                               |
|                |                      | NCT03795610      | Phase 2    | Head and neck squamous cell carcinoma                                        |
| Brutinib       | BTK                  | Approved         |            |                                                                            |
| Plexixafor (AMD3100) | CXCR4              | Approved         |            |                                                                            |
| SX-682         | Dual CXCR1/2         | NCT04599140      | Phase 1/2  | Metastatic colorectal cancer                                                |
|                |                      | NCT04574583      | Phase 1/2  | Advanced solid tumors                                                       |
|                |                      | NCT04477343      | Phase 1    | Metastatic pancreatic duct adenocarcinoma                                  |
|                |                      | NCT03161431      | Phase 1    | Melanoma                                                                    |
|                |                      | NCT04245397      | Phase 1    | Myelodysplastic syndromes                                                   |
| AZD5069        | CXCR2                | NCT03177187      | Phase 1/2  | Metastatic castration resistant prostate cancer                             |
|                |                      | NCT02499328      | Phase 2    | Advanced solid tumors, metastatic head and neck squamous cell carcinoma     |
|                |                      | NCT02583477      | Phase 1/2  | Metastatic pancreatic duct adenocarcinoma                                  |
| X4P-001        | CXCR4                | NCT02823405      | Phase 1    | Melanoma                                                                    |
|                |                      | NCT02923531      | Phase 1/2  | Clear cell renal cell carcinoma                                             |
|                |                      | NCT02667886      | Phase 1/2  | Clear cell renal cell carcinoma                                             |
| Maraviroc      | CCR5                 | NCT01785810      | Phase 2    | Metastatic colorectal cancer                                                |
|                |                      | NCT03274804      | Phase 1    | Colorectal cancer                                                           |
| BMS-813160     | Dual CCR2/5          | NCT03184870      | Phase 1/2  | Colorectal cancer, pancreatic cancer                                         |
|                |                      | NCT04123379      | Phase 2    | Non-small cell lung cancer, hepatocellular carcinoma                         |
|                |                      | NCT02996110      | Phase 2    | Advanced renal cell carcinoma                                               |
|                |                      | NCT03767582      | Phase 1/2  | Locally advanced pancreatic ductal adenocarcinomas                         |
|                |                      | NCT03496662      | Phase 1/2  | Pancreatic ductal adenocarcinoma                                            |
| FLX-475        | CCR4                 | NCT03674567      | Phase 1/2  | Advanced cancer                                                             |

Data were collected from ClinicalTrials.gov. Abbreviations: PD-L1, programmed death protein-ligand 1; VISTA, V-domain Ig suppressor of T cell activation; NCT, clinicaltrials.gov identification number; TLR, toll-like receptor; IDO1, indoleamine-2,3-dioxygenase-1; ARG, arginase; A2A, Adora2a; STING, stimulator of interferon genes; CSF1R, colony stimulating factor 1 receptor; RORγt, receptor-related orphan receptor gamma t; SHP2, Src homology-2-containing protein tyrosine phosphatase 2; PI3K, phosphoinositide-3 kinase; BTK, Brutons tyrosine kinase; CXCR, C-X-C chemokine receptor; CCR, C-C chemokine receptor.
occurrence of irAEs must be anticipated and monitored. The advantages of small molecules are permeabilization, oral delivery, and dose modulation, which promote the development of small molecule inhibitors acting on the PD-1/PD-L1 pathway [5–7].

Companies Bristol-Myers Squibb (BMS) and Aurigene are leading the development of small molecule PD-L1 inhibitors, with molecules such as BMS-103, BMS-142, BMS-1166, CA-327, and CA170. Small PD-L1 inhibitors developed by BMS can induce the PD-L1 dimer by filling a deep hydrophobic channel-like pocket between two PD-L1 molecules and then blocking PD-1 binding [8,9]. Oral molecule, CA-327, shows anti-tumor activity in preclinical cancer models by inhibiting the PD-L1 and T cell immunoglobulin domain and mucin domain-3 (TIM-3) [10].

Developed by Aurigene, CA170 is an oral inhibitor that targets PD-L1 and the V-domain Ig suppressor of T cell activation (VISTA), and was reported as the pioneer of oral immunotherapy drugs among small molecule checkpoint inhibitors [11]. A CA-170 phase 2 clinical study is currently ongoing with data obtained from 15 non-small cell lung cancer cases and notable tumor reductions noted in six patients [12]. However, the affinity of small molecules to the target is worse than that of antibodies. Hence, off-targeting may occur and result in reduced efficacy and toxicity. Further mechanism explorations and clinical efficacy evaluations are needed. Although small molecule immune checkpoint inhibitors are mostly in preclinical and early clinical stages, these drugs will open a new avenue for tumor immunotherapy because of their pharmacokinetics and druggability advantages.

Targeting innate immunity

Pattern recognition receptors are key members in innate immunity that can distinguish pathogen-associated molecular patterns and promote T cell effector function [13]. Toll-like receptor (TLR) 7/8 is located in the endosome of cells. By improving the identification of foreign organisms, small molecule TLR agonists activate immune response. Imiquimod, a TLR7 agonist developed as topical cream by the Minnesota Mining & Manufacturing Company (the United States) has been used for superficial basal cell carcinoma [14]. This drug has also shown anti-tumor activity in a phase 2 clinical trial for patients with bladder

---

**Fig. 1** Small molecule drugs and their targets in immunotherapy. This figure was created with BioRender.com. ATP, adenosine triphosphate; STING, stimulator of interferon genes; TLR, toll-like receptor; A2A, Adora2a; MDSCs, myeloid-derived suppressor cells; CXCR, C-X-C chemokine receptor; ARG1, arginase 1; IDO1, indoleamine-2,3-dioxygenase-1; PD-1, programmed death protein 1; PD-L1, programmed death protein-ligand 1; SHP2, Src homology-2-containing protein tyrosine phosphatase 2; SHIP1, SH2 domain-containing inositol-5′-phosphatase 1; PI3K, phosphoinositide-3 kinase; BTK, Brutons tyrosine kinase; RORγt, receptor-related orphan receptor gamma t; TAMs, tumor-associated macrophages; CSFR1, colony stimulating factor 1 receptor.
cancer [15]. Motolimod (VTX-2337), an agonist of TLR8, can mediate the release of IL-18 and activate natural killer (NK) cells [16]. Resiquimod (R848), a TLR7/8 agonist, helps macrophages acquire an anti-tumorigenic phenotype [17]. These TLR7/8 agonists are mostly in phase 1/2 clinical trials (Table 1).

Stimulator of interferon genes (STING) participate in the innate immune recognition of immunogenic tumors [18]. The activation of the STING pathway contributes to tumor regression in mouse models [19]. STING agonists might also improve the activation of dendritic cells (DCs) and T cells [20]. In June of 2019, Aduro announced the results of a phase 1b clinical trial for a small molecule STING antagonist (ADU-S100) combined with spartalizumab. However, only 6 out of the 83 patients with lymphoma or advanced solid tumors exhibited remarkable responses [21]. In hope of achieving relatively improved results, Aduro is currently preparing to combine ADU-S100 and Keytruda for head and neck cancers in a phase 2 clinical trial. The STING small molecule antagonist MK-1454 is also in a phase 2 clinical trial (NCT04220866).

In addition to antibodies for checkpoint modulation and cell therapy, pattern recognition receptor agonists and STING agonists provide a new approach for small molecules to prompt innate immune members to contribute to anti-tumor immune strategies. Although TLR agonists are promising targets that may exhibit synergistic effects with existing immunotherapy strategies, future research must consider that the TLR pathway is associated with gastric and pancreatic tumorigenesis [22,23]. Additional studies are required to further assess the safety of these small molecule agonists.

**Targeting amino acid metabolism**

The TME contains diverse immunocytes. Tumor-associated macrophages (TAMs) support tumor invasion and metastasis. Treg cells and myeloid-derived suppressor cells (MDSCs) are linked to immunosuppression in the TME. Small molecule drugs navigating metabolic pathways might strengthen the anti-tumor immunity by metabolic reprogramming of tumor and immune cells in the TME [24].

Indoleamine-2,3-dioxygenase-1 (IDO1) participates in the degradation of tryptophan to kynurenine, and selective inhibition of IDO1 enhances NK cell proliferation and reduces conversion to Treg cells [25]. BMS-986205 is one highly-efficient oral IDO1 inhibitor that can shrink bladder tumors when combined with ICIs from a phase 1/2a study [26]. IDO1 inhibitor navoximod has also shown acceptable safety and tolerance in a phase 1 clinical trial of advanced solid tumors, but its combination with atezolizumab was not beneficial [27]. A recent phase 3 trial, ECHO301, tested the efficacy of IDO1 inhibitor epacadostat combined with pembrolizumab in melanoma; however, the reaction was not better than that for pembrolizumab alone [28].

Small molecule arginase 1 (ARG1) or inducible nitric oxide synthase (iNOS) inhibitors targeting MDSCs or TAMs might overcome immunosuppression and aid the restoration of immune function [29]. ARG1 inhibitor CB-1158 promotes the production of inflammatory cytokines and increases CD8+ T cell tumor infiltration [30]. CB-1158 is now under phase 1/2 clinical trials and is also being combined with a small molecule PD-1 blockade (Table 1). Transient treatment with CB-839, an inhibitor of glutaminase 1, also enhances cytotoxic lymphocyte-mediated anti-tumor responses [31].

Treatments targeting the amino acid metabolism of tumor and/or immune cells in the TME can produce a synergistic effect with existing immunotherapy approaches. However, the unexpected efficacy of IDO1 inhibitor epacadostat combined with pembrolizumab in clinical trial suggests that much efforts are need to further understand the metabolic mechanisms of immune cells to improve the effectiveness of combination therapies.

**Targeting adenosine signaling**

Ectonucleotidases CD73 and CD39 participate in the dephosphorylation of adenosine triphosphate to produce adenosine, which binds to the Adora2a (A2A) receptor, activates adenosine signaling, and amplifies the immunosuppressive effects of Treg cell [32]. In preclinical studies, the efficacy of ICIs have been enhanced using a combination of A2A receptor antagonists [33]. Preliminary evidence from a phase 1b clinical trial showed that A2A receptor inhibitor CPI-444 combined with atezolizumab exhibits disease control in refractory renal cell carcinoma [34]. Other phase 1/2 studies have also assessed the safety of A2A receptor antagonists used alone or combined with ICIs in advanced tumors (Table 1). Given the immunosuppressive role of adenosine signaling in the TME, small molecule antagonists targeting A2A receptor show potential as therapeutics.

**Targeting cytokine signaling**

Small molecules can regulate the tumor immune response by influencing specific cytokine-mediated pathways. Retinoic acid receptor-related orphan receptor gamma t (RORγt) is a member of the nuclear receptor superfamily of transcription factors and plays an important role in the differentiation of cytokine interleukin-17 expressing immune cells [35]. RORγt agonists enhance anti-tumor immunity by activating Th17 cells and reducing Treg proliferation [36]. RORγt agonist, LYC-55716 in
combination with an ICI, is currently undergoing a phase 1 clinical trial (Table 1).

Galunisertib, a transforming growth factor-beta (TGF-β) receptor 1 inhibitor, suppresses Smad family member 2 phosphorylation and was granted orphan drug designation for the treatment of liver cancer by the European Medical Agency and the FDA in the United States in 2013 [37]. Galunisertib combined with a PD-L1 blockade can enhance the expression of immune-related genes and modulate T cell immunity in colorectal and breast cancer mouse models [38].

Although the relationship between these cytokines and immune regulation has been established, only a few of these drugs are currently undergoing clinical trials, possibly because they mediate complex signaling pathways. Their effects on tumor cells and immune cells in the TME and the risks of combination drugs must be paid attention.

**Targeting oncogenic phosphatases and kinases**

Phosphatases and kinases that regulate signal transduction are potential targets for small molecule drugs. Src homology-2-containing protein tyrosine phosphatase 2 (SHP2) is involved in the downstream signaling of PD-1, which suppresses T cell function [39]. Owing to its crucial role in T cell activation, SHP2 has emerged as a treatment strategy. In colon cancer xenograft models, SHP2 inhibitor SHP099 combined with an anti-PD-1 antibody showed better reducing ability for tumor load than monotherapy [40]. The SHP2 inhibitor RMC-4630s is currently under phase 1/2 clinical trials, and its pharmacokinetic profile and safety are also being evaluated (Table 1).

Colony stimulating factor 1 receptor (CSF1R) is activated by phosphorylation; pexidartinib, an oral CSF1R inhibitor, decreases TAMs and increases CD8+ T cells when used in combination with a dendritic cell cancer vaccine in mesothelioma mouse models [41]. Two clinical trials of pexidartinib monotherapy and two clinical trials of pexidartinib combined with monoclonal antibodies in advanced tumors are currently ongoing.

3-α-Aminocholestane, a small molecule inhibitor of lipid phosphatases SH2 domain-containing inositol-5’-phosphatase 1 (SHIP1), can strengthen the antitumor response of NK and T cells in mouse models [42]. IPI-549, a phosphoinositide-3 kinase (PI3K)-γ inhibitor, can inhibit neutrophil migration and increase the antitumor efficacy of CD8+ T cells [43,44]. IPI-549 used alone or in combination with ICIs is currently under investigation (Table 1). Ibrutinib, an inhibitor of Brutons tyrosine kinase (BTK), can also enhance T cell function in leukemia [45].

These small molecule drugs targeting phosphorylases and kinases usually affect tumor cell signal transduction. Additional research is needed to clarify their overall influence on tumor and immune cells prior to clinical trials.

**Targeting chemokine receptors**

The chemokine superfamily consists of a large number of ligands and receptors that participate in the homing, retention, circulation, and activation of immune cells [46]. C-C chemokine receptor (CCR) 2 inhibitor PF-04136309 depletes macrophages and inflammatory monocytes from the primary lesion and premetastatic liver, thereby enhancing antitumor immunity, depressing tumor growth, and reducing metastasis [47]. Inhibiting C-X-C chemokine receptor (CXCR) 4 may also reduce the accumulation of macrophages in the TME [48]. Plerixafor, a CXCR4 antagonist, has achieved good results as a chemosensitizer in phase 1/2 leukemia clinical trials [49]. Other ongoing clinical trials of small molecule drugs targeting chemokine receptors have focused on CCR2/5 antagonist BMS-813160, CCR4 inhibitor FLX475, and CXCR2 antagonist AZD5069 (Table 1).

The small molecule targeting of chemokine receptors is often used in combination with ICIs and chemotherapeutics in clinical trials. Given the important role of chemokines in the TME, the combinational strategies may provide meaningful clinical benefits. At present, numerous small molecule drugs have been developed to target the extracellular or intracellular pathways in adaptive or innate immunity; however, most of them are in the early stage of clinical trials. Additional basic experiments and clinical trials are urgently required to clarify their mechanism, clinical efficacy, and pharmacokinetics.

**Bispecific antibodies (bsAbs)**

**An overview**

First described in the 1960s [50], bsAbs are special molecules that can bind two antigens or one antigen with different epitopes. The technological innovation of bsAbs subsequently developed in antibody engineering and biology (Fig. 2) [51]. At present, only three bsAbs are approved for global marketing: catumaxomab (CD3/EpCAM) [52], blinatumomab (CD3/CD19) [53], and emicizumab (FIXa/FX or Hemlibra) [54].

BsAbs are utilized in various ways, including receptor-activation, receptor-blocking, receptor-internalization, receptor-clustering, or retargeting of cytotoxic effector cells [55]. Cancer is a complicated and polyfactorial disease. Compared with monospecific monoclonal antibodies, bsAbs can synchronously bind two individual epitopes or antigens for greater impact and better treatment effects. Multi-combined regions in one antibody could help regulate diverse functional pathways in cancer, thus
avoiding drug resistance and decreasing the side effects on intravital tissues [56–59].

With the rapid development of gene engineering antibodies and immunology, the construction, technology platform, product research, and development of bsAbs are continuously being innovated at high speed. BsAbs are expected to be the next generation of biological therapeutics for tumors, autoimmune illness, contagious diseases, diabetes, Alzheimer’s disease, and osteoporosis [51,60]. However, several challenges have been encountered during their development, namely, how to prevent poisoning and immunogenicity due to neo-antigenic determinants, how to meet the threshold for sensitizing diverse molecular mechanisms, and how to ensure the manufacturing quality [61].

Preparation method of bsAbs

BsAbs contain two different antigen binding domains that cannot be found in nature and can only be prepared artificially. Chemical coupling [62], two-hybrid method [63], and genetic engineering [64] are the most common preparation techniques for bsAbs. The most attractive application is the realization of new biological functions and therapeutic mechanism of action (MOA). However, new MOAs pose undiscovered risks that cannot be estimated in preclinical research. The indeterminacy over their safety is the major hurdle in the exploration of bsAbs. Molecular imaging studies could be used to create predictive models for the pharmacokinetic parts of bsAbs and to develop optimal dosing strategies [65].

Structure types

The basic structure of bsAbs consists of two pairs of heavy-light polypeptide chains connected by interchain disulfide and noncovalent bonds resembling a “Y” shape compound, including antigen binding fragments (Fab) and a fragment crystallizable region (Fc). BsAbs could help immune cells target tumor cells by binding to one surface antigen expressed on cancer cells and to a second antigen expressed on immune cells, such as NK cells or effector T cells. The fusing of the antitumor binding domain with the Fc receptor (FcR) or the anti-CD3 binding domain may help produce bsAbs that can recruit immune cells. FcR is the terminal area of the antibody that interplays with the neonatal receptor, which results in

Fig. 2 Timeline of the conceptual and technical innovations contributing to the therapeutic bsAb landscape. bsAb, bispecific antibodies; DVD, dual variable domain; EU, European Union; Fab, antigen binding fragment; HC, heavy chain; Ig, immunoglobulin; LC, light chain; scFv, single-chain variable fragment.
lethal immune-mediated effects [66,67].

BsAbs can be divided into two categories according to their structure: one contains the Fc region, and the other lacks the Fc region. These types can be further classified into asymmetric IgG-like bsAbs, symmetric IgG-like bsAbs, and non-IgG-like bsAbs [68]. IgG-like bsAbs can achieve effector functions, and non-IgG-like binding antibody (bAbs) are diminutive, which can improve penetration. IgG-like bAbs contain three arms/binding sites: two Fab arms and an Fc arm. The IgG-like bsAb structure promotes Fc domain-mediated effects and defends the physical properties endowed by the FcR [69,70]. A unique kind is asymmetric IgG-like bsAbs that possess an integrated Fc and a couple of distinguishing arms combining different antigens; some examples include M802, M701 [51], KN026 [71], MBS301 [72], IBI318 [73], IBI315 [74], and KN046 [75].

Symmetric IgG-like bsAbs are composed of an IgG-like Fc and a pair of symmetric arms formed by the association between different Fabs, single-chain variable fragment (scFV), and variable domain of heavy chain (VHH); these include EMB-01 [76], ES101 [77], K193 [78], AK104 [79], SI-B001 [80], and MGD013 [81]. Non-IgG-like bsAbs lack the Fc domain and exert the corresponding effect mechanism mainly through the characteristics of antigen binding; these include SHR-1701 [82], IMM0306 [83], and HX009 [84].

Mechanisms of bsAbs

BsAbs have manifold targets and special MOA.

T cell redirection

BsAbs characteristically target the antigen connected to T cells. By bonding to T cells and cancer cells, they can redirect the toxicity of effector T cells and obliterate cancer cells [85,86].

Double checkpoint inhibition

BsAbs can block PD-1 or lymphocyte-activation gene 3 (LAG3), PD-L1, TIM-3, cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), and T cell immunoreceptor with immunoglobulin and ITIM domain (TIGIT) interaction, thereby activating tumor immune response [87–90]. A number of current clinical trials are targeting the above two immune checkpoints [91–94].

Co-localized blockage

SHR-1701 can simultaneously block the PD-L1 immune checkpoint and TGF-β on cancer cells. The aforementioned combination therapy could also increase the antineoplastic effect compared with mono-treatment in cancer cell pathways [95,96].

Dual signaling inhibitions

EMB-01 (EGFR/MET) has shown promising effectiveness in numerous preclinical tests. EGFR and MET signaling paths are partly complementary and mediate the restriction of signal pathways [97–99]. SI-B001 (EGFR/HER3) activates the downstream pathways and inhibits tumorigenesis [100,101].

Tumor targeted immune-modulators

Tumor-targeted immune-modulators are intended to be combined with tumor-associated antigen (TAA) and immune-regulating receptors (PD-1/CD47) to improve immune-treatment by orientating cancer cells. Such modulators include IBI315(HER2/PD-1) [74] and IMM0306 (CD47/CD20) [102].

Biparatopic bsAbs (bpAbs)

BpAbs combine two non-overlapping sites of identical antigen to cement Ab-Ag reciprocity and enhance the cancer cellular targeting of monoclonal antibodies [103]; these include KN026 (HER2/HER2) [104] and MBS301 (HER2/HER2) [105].

Research status

Many multinational pharmaceutical companies and biotechnology companies have committed to developing bsAb-related drugs. Many Chinese companies are also involved in the research and development of bsAbs, some of which have entered the clinical or clinical application stage.

More than 100 bsAb constructions and 200 clinical trials and over 30 technology research platforms, including CrossMab (Roche), CRIBTM (China), ItabTMv (China), and FIT-IgTM (China), have been conducted over the past decade [61]. Despite starting later than other countries, Chinese bsAb development has rapidly progressed. By using the aforementioned bsAb technology research platforms, China has created 18 bsAb structures and initiated 25 clinical trials (Table 2). As of August 2020, PD-L1 and CTLA-4 are the most commonly studied targets in China [106]. In particular, 10 bsAb and 41 clinical trials were noted for both China and other countries (Table 3) [106]. 90 bsAb structures and 149 clinical trials are currently being studied outside of China (Table 4).

AK104 (PD-1/CTLA-4) is under a recent phase 2 multicenter study on advanced gastric adenocarcinoma. The common targeted cancer bsAb simultaneously blocks the
PD-1 and CTLA-4 immune regulatory checkpoints, resulting in the potential suppression of double checkpoints and antineoplastic activity [107]. Developed by Alphamab, KN046 (PD-L1/CTLA-4) is currently in a phase 3 trial. Some studies have recently reported treatment-related toxic side effects of anti-CTLA-4 antibody [108,109]. Compared with each parental mAbs, KN046 can improve the safety and efficacy [110].

Developed by Biokin, SI-B001 is an anti-HER3 × anti-EGFR bsAb that is currently in a phase 1 trial and could firsthand activate the downstream paths and inhabit tumorigenesis [100,101].

BsAbs have great clinical potential because of their unique characteristics that cannot be found in monoclonal antibodies. Most bsAbs are in clinical or preclinical research. Adverse reactions, such as cytokine storms, neurotoxicity, and production processing, are the main problems for this therapy. Designing a reasonable antibody structure according to different effect mechanisms is the focus of bsAb research and development. The continued development of clinical studies and advances in upstream and downstream technology will hopefully help to solve these bsAb-related problems.

### Chimeric antigen receptor (CAR) T cell therapy

CAR T cells are T cells designed to express an artificial receptor that redirects the T cell toward tumor cell antigen. CAR T cell therapy is one of the most encouraging therapeutic strategies and has remarkable clinical potential. CARs are composed of four domains including the extracellular domain, the transmembrane (TM) domain, the intracellular domain, and an activation domain. The first-generation of CARs comprise an extracellular domain

| Antibody name | Targets | Clinical studies | Phase | Cancer type |
|---------------|---------|-----------------|-------|-------------|
| MBS-301 | HER2 × HER2 | NCT03842085 | Phase 1 | Her2 positive recurrent or metastatic malignant solid tumor |
| IBI-318 | PD-1 × PD-L1 | NCT03875157 | Phase 1 | Advanced malignancy |
| IBI-322 | PD-L1 × CD47 | NCT04338659 | Phase 1 | Advanced malignancies |
| IBI-315 | PD-1 × HER2 | NCT04162327 | Phase 1 | Advanced solid tumor |
| A-319 | CD3 × CD19 | NCT04056975 | Phase 1 | Relapsed or refractory B cell lymphoma |
| M701 | CD3 × EpCAM | NCT04501744 | Phase 1 | Malignant ascites |
| M802 | HER2 × CD3 | NCT04501770 | Phase 1 | Her2 positive advanced solid tumor |
| IMM0306 | CD47 × CD20 | CTR20192612 | Phase 1 | Refractory or recurrent CD20 positive B cell non-Hodgkin’s lymphoma |
| KN-026 | HER2 × HER2 | CTR20190853 | Phase 2 | Her2 positive advanced solid tumor |
| EMB-01 | EGFR × c-MET | CTR20190241 | Phase 2 | Advanced or metastatic solid tumors |
| KN-046 | PD-L1 × CTLA-4 | NCT04469725 | Phase 2 | Thymic carcinoma |
| AK-104 | PD-1 × CTLA-4 | CTR20182027 | Phase 1/2 | Advanced solid tumor and advanced or metastatic gastric adenocarcinoma or gastroesophageal junction adenocarcinoma |
| MGD-013 | PD-1 × LAG-3 | NCT04009460 | Phase 1 | Solid tumors |
| HX-009 | PD-1 × CD47 | CTR20200549 | Phase 2 | Advanced hepato cellular carcinoma |
| M7824 | PD-L1 × TGF-β | NCT04396886 | Phase 2 | Recurrent or metastatic carcinoma |
| SHR-1701 | PD-L1 × TGF-β | CTR20182404 | Phase 1 | Advanced solid tumor |
| SI-B001 | HER3 × EGFR | CTR20200502 | Phase 1 | Locally advanced or metastatic epithelial tumors |
| K193 | CD3 × CD19 | CTR20191955 | Phase 1 | Refractory or recurrent B cell non-Hodgkin’s lymphoma |

Abbreviations: HER, human epidermal growth factor receptor; NCT, clinicaltrials.gov identification number; PD-1, programmed death protein 1; PD-L1, programmed death protein-ligand 1; CTR, Clinical Trial Registry; EpCAM, epithelial cell adhesion molecule; c-MET, cellular-mesenchymal epithelial transition; CTLA-4, cytotoxic T lymphocyte-associated antigen-4; LAG-3, lymphocyte-activation gene 3; TGF, transforming growth factor; EGFR, epidermal growth factor receptor.
linked to an intracellular domain without any co-stimulatory domain. However, no promising antitumor response was observed largely due to the lack of adequate activation [111]. As a solution, second- and third-generation CARs are being developed by adding one or two co-stimulatory domains, respectively, to enhance their activity [112,113].

Second-generation autologous (patient-derived) CAR T cell therapy has changed the treatment of hematologic malignancies; four CD19-targeting CARs have achieved

| Antibody name | Targets | Clinical studies | Phase | Cancer type |
|---------------|---------|------------------|-------|-------------|
| KN-026        | HER2 × HER2 | NCT0166993      | Phase 2 | Metastatic breast cancer |
|               |         | NCT03847168     | Phase 1 | Breast cancer |
|               |         | NCT04046999     | Phase 1 | Her2 positive solid tumors |
|               |         | NCT03619681     | Phase 1 | Breast cancer, gastric cancer |
|               |         | NCT03925974     | Phase 2 | Gastric, garesophageal junction cancer |
| EMB-01        | EGFR × c-MET | NCT03797391  | Phase 1/2 | Neoplasm metastasis, non-small cell lung cancer |
| JNJ-61186372, JNJ-6372 | EGFR × c-MET | NCT02609776 | Phase 1 | Non-small cell lung cancer |
|               |         | NCT04077463     | Phase 1 | Carcinoma, non-small-cell lung |
| KN-046        | PD-L1 × CTLA-4 | NCT04040699 | Phase 1 | Her2 positive solid tumors |
|               |         | NCT03838848     | Phase 2 | Advanced non-small cell lung cancer |
|               |         | NCT03927945     | Phase 2 | Esophageal squamous cell carcinoma |
|               |         | NCT03925870     | Phase 2 | Esophageal squamous cell carcinoma |
|               |         | NCT03733951     | Phase 1 | Advanced solid tumors |
|               |         | NCT04054531     | Phase 2 | Non-small cell lung cancer |
|               |         | NCT03872791     | Phase 1/2 | Triple-negative breast cancer |
|               |         | NCT03529526     | Phase 1 | Advanced solid tumors |
| AK-104        | PD-L1 × CTLA-4 | NCT04380805 | Phase 2 | Recurrent or metastatic cervical cancer |
|               |         | NCT04172454     | Phase 1/2 | Advanced solid tumors |
|               |         | NCT04220307     | Phase 2 | Nasopharyngeal carcinoma |
|               |         | NCT03261011     | Phase 1 | Advanced cancer |
|               |         | NCT03852251     | Phase 1/2 | Advanced solid tumors |
| MGD-013       | PD-L1 × LAG-3 | NCT04212221 | Phase 1/2 | Advanced hepatocellular carcinoma |
|               |         | NCT03219268     | Phase 1 | Advanced solid tumors |
|               |         | NCT04178460     | Phase 1 | Gastric cancer |
|               |         | NCT04082364     | Phase 2/3 | Her2 positive gastric cancer, breast cancer |
| INBRX-105-1, INBRX-105, ES-101 | PD-L1 × 4-1BB | NCT03809624 | Phase 1 | Metastatic solid tumors |
|               |         | NCT04009460     | Phase 1 | Solid tumors |
| HX-009        | PD-L1 × CD47 | NCT04097769     | Phase 1 | Advanced solid tumors |
| M7824         | PD-L1 × TGF-β | NCT04246489  | Phase 2 | Uterine cervical neoplasms |
|               |         | NCT04066491     | Phase 2/3 | Biliary tract cancer |
|               |         | NCT04396353     | Phase 2 | Advanced lung non-small cell carcinoma |
|               |         | NCT04220775     | Phase 1/2 | Recurrent head and neck squamous cell carcinoma |
|               |         | NCT03631706     | Phase 3 | Non-small cell lung cancer |
|               |         | NCT02517398     | Phase 1 | Solid tumors |
|               |         | NCT03840915     | Phase 1/2 | Non-small cell lung cancer |
|               |         | NCT03840902     | Phase 2 | Non-small cell lung cancer |
|               |         | NCT03833661     | Phase 2 | Biliary tract cancer |
| SHR-1701      | PD-L1 × TGF-β | NCT03710265 | Phase 1 | Solid tumors |
|               |         | NCT03774979     | Phase 1 | Solid tumors |
|               |         | NCT04282070     | Phase 1 | Nasopharyngeal carcinoma |
|               |         | NCT04324814     | Phase 1 | Advanced solid tumors |

Abbreviations: HER, human epidermal growth factor receptor; NCT, clinicaltrials.gov identification number; EGFR, epidermal growth factor receptor; c-MET, cellular-mesenchymal epithelial transition; PD-L1, programmed death protein-ligand 1; CTLA-4, cytotoxic T lymphocyte-associated antigen-4; PD-1, programmed death protein 1; LAG-3, lymphocyte-activation gene 3; TGF, transforming growth factor.
### Table 4  BsAbs under clinical development excluding China as of August 2020

| Antibody name | Targets | Clinical studies | Phases | Cancer type |
|---------------|---------|------------------|--------|-------------|
| Dilpacimab, ABT-165 | VEGF × DLL4 | NCT01946074 | Phase 1 | Advanced solid tumors |
| | | NCT03368859 | Phase 2 | Neoplasms |
| MP0250 | VEGF × HGF | NCT03136653 | Phase 1/2 | Relapsed multiple myeloma |
| | | NCT03418532 | Phase 1/2 | EGFR positive lung cancer |
| | | NCT02194426 | Phase 1/2 | Neoplasms |
| ABL-001, NOV-1501, TR-009 | VEGF × DLL4 | NCT02857868 | Phase 1 | Neoplasms |
| | | NCT03595917 | Phase 1 | Chronic myeloid leukemia, acute lymphoblastic leukemia |
| | | NCT03106779 | Phase 3 | Chronic myelogenous leukemia |
| | | NCT04216563 | Phase 2 | Philadelphia chromosome negative, BCR-ABL1 positive chronic myelogenous leukemia |
| | | NCT03292783 | Phase 1 | Advanced solid tumors |
| | | NCT02081378 | Phase 1 | Chronic myelogenous leukemia, Philadelphia chromosome-positive acute lymphoblastic leukemia |
| Vanucizumab, RG-7221 | ANGPT2 × VEGF | NCT01688206 | Phase 1 | Neoplasms |
| | | NCT02141295 | Phase 2 | Colorectal cancer |
| | | NCT02665416 | Phase 1 | Advanced or metastatic solid tumors |
| BI-836880 | ANGPT2 × VEGF | NCT02689505 | Phase 1 | Neoplasms |
| | | NCT02674152 | Phase 1 | Neoplasms |
| | | NCT03972150 | Phase 1 | Neoplasms |
| | | NCT03861234 | Phase 1 | Neoplasms |
| | | NCT03468426 | Phase 1 | Non-squamous, non-small-cell lung cancer, neoplasms |
| Navicixizumab, OMP-305B83 | VEGF × DLL4 | NCT03035253 | Phase 1 | Metastatic colorectal cancer |
| | | NCT03030287 | Phase 1 | Ovaries cancer, fallopian tube cancer |
| | | NCT02298387 | Phase 1 | Advanced solid tumor malignancies |
| ZW-25 | HER2 × HER2 | NCT04224272 | Phase 2 | Her2 or HR positive breast cancer |
| | | NCT02892123 | Phase 1 | Her2 positive cancers |
| | | NCT03929666 | Phase 2 | Her2 positive gastroesophageal adenocarcinoma |
| | | NCT04276493 | Phase 1/2 | Breast cancer, gastric cancer, gastroesophageal junction cancer |
| MCLA-128 | HER2 × HER3 | NCT03321981 | Phase 2 | Metastatic breast cancer |
| | | NCT02912949 | Phase 1/2 | Harboring NRG1 fusion solid tumors |
| BCD-147 | HER2 × HER2 | NCT03912441 | Phase 1 | Neoplasms |
| BI-905677 | LRP5 × LRP6 | NCT03604445 | Phase 1 | Neoplasms |
| MP0274 | HER2 × HER2 | NCT03084926 | Phase 1 | Neoplasms |
| DuoBody-PD-L1x4-1BB, GEN-1046 | PD-L1 × 4-1BB | NCT03917381 | Phase 1/2 | Solid tumors |
| REGN-5678 | CD28 × PSMA | NCT03972657 | Phase 1/2 | Metastatic castration-resistant prostate cancer |
| FS118 mAb2, FS-118, LAG-3/PD-L1 mab2 | PD-L1 × LAG-3 | NCT03440437 | Phase 1 | Advanced cancer |
| LY-3434172 | PD-1 × PD-L1 | NCT03936959 | Phase 1 | Advanced cancer |
| XmAb-23104 | PD-1 × ICOS | NCT03752398 | Phase 1 | Advanced solid tumors |
| ABBV-428 | MSLN × CD40 | NCT02955251 | Phase 1 | Advanced solid tumors |
| ADC-1015, ATOR-1015 | OX40 × CTLA-4 | NCT03782467 | Phase 1 | Solid tumor |
| MCLA-145 | PD-L1 × 4-1BB | NCT03922204 | Phase 1 | Advanced solid tumor, B cell lymphoma |
| MEDI-5752 | PD-1 × CTLA-4 | NCT03530397 | Phase 1 | Selected advanced solid tumors |
| MGD-019 | PD-1 × CTLA-4 | NCT03761017 | Phase 1 | Advanced solid tumor |
| PRS-343 | HER2 × 4-1BB | NCT03330561 | Phase 1 | Her2 positive solid tumor |
| | | NCT03650348 | Phase 1 | Her2 positive solid tumor |
| Antibody name | Targets | Clinical studies | Phases | Cancer type |
|---------------|---------|-----------------|--------|-------------|
| RG-7769, RO-7T21661 | PD-1 × TIM-3 | NCT03708328 | Phase 1 | Solid tumors |
| XmAb-20717 | PD-1 × CTLA-4 | NCT03517488 | Phase 1 | Solid tumors |
| XmAb-22841 | CTLA-4 × LAG-3 | NCT03849469 | Phase 1 | Solid tumors |
| MP0310 | FAP × CD40 | NCT04049903 | Phase 1 | Advanced solid tumor |
| AK-112 | VEGF × PD-1 | NCT04047290 | Phase 1 | Neoplasms malignant |
| GEN-1042 | CD40 × 4-1BB | NCT04383599 | Phase 1/2 | Solid tumor, non-small cell lung cancer, colorectal cancer, melanoma |
| AGEN-1423, GS-1423 Tebentafusp (IMCgp100) | CD73 × TGF-β gp100/HLA-A*0201 × CD3 | NCT03954704 | Phase 1 | Advanced solid tumors |
| OXS-1550, DT-2219 | CD19 × CD22 | NCT02370160 | Phase 1/2 | Refractory or relapsed B-lineage leukemia |
| AFM-13 | CD16 × CD30 | NCT02321592 | Phase 2 | Hodgkin lymphoma |
| Odrontemab, REGN-1979 | CD3 × CD20 | NCT02651662 | Phase 1 | Lymphoma |
| IMC-C103C | MAGE-A4/HLA *A0201 × CD3 | NCT03973333 | Phase 1/2 | Advanced solid tumors |
| IMCnyeso | NY-ESO-1/HLA *A0201 × CD3 | NCT03515551 | Phase 1/2 | Advanced solid tumors |
| Mosunetuzumab, RG-7828 | CD3 × CD20 | NCT03671018 | Phase 1/2 | B cell non-Hodgkin lymphoma |
| OXS-3550, CD161533 TriKE | CD16 × CD33 | NCT03214666 | Phase 1/2 | Acute myelogenous leukemia, mast cell leukemia |
| GEN-3013 | CD3 × CD20 | NCT03625037 | Phase 1/2 | Lymphoma |
| MCLA-117 | CD3 × CLEC12 | NCT0308230 | Phase 1 | Acute myelogenous leukemia, acute myeloid leukemia |
| Flotetuzumab, MGD-006 | CD3 × CD123 | NCT03739606 | Phase 2 | Acute and chronic myelogenous leukemia |
| MGD-007 | CD3 × GPA33 | NCT03531632 | Phase 1/2 | Colorectal cancer metastatic |
| REGN-4018 | CD3 × MUC16 | NCT03564340 | Phase 1/2 | Recurrent ovarian cancer, recurrent fallopian tube cancer, recurrent primary peritoneal cancer |
| Cibisatamab, RO-6958688, RG-7802 | CD3 × CEA | NCT02650713 | Phase 1 | Solid tumors |

(Continued)
| Antibody name | Targets          | Clinical studies | Phases   | Cancer type                                                                 |
|---------------|------------------|------------------|----------|-----------------------------------------------------------------------------|
| AMG-701       | CD3 \times BCMA  | NCT03287908      | Phase 1  | Relapsed or refractory multiple myeloma                                     |
| AMG-160       | CD3 \times PSMA  | NCT03792841      | Phase 1  | Metastatic castration-resistant prostate cancer, prostate cancer            |
| AMG-330, MT-114 | CD3 \times CD33 | NCT02520427      | Phase 1  | Relapsed or refractory acute myeloid leukemia                               |
| AMG-424       | CD3 \times CD38  | NCT03455663      | Phase 1  | Relapsed or refractory multiple myeloma                                     |
| AMG-427       | CD3 \times FLT3  | NCT03541639      | Phase 1  | Relapsed or refractory acute myeloid leukemia                               |
| AMG-562       | CD3 \times CD19  | NCT03571828      | Phase 1  | Diffuse large B cell lymphoma, mantle cell lymphoma, follicular lymphoma    |
| AMG-596       | CD3 \times EGFRvIII | NCT03296606  | Phase 1  | Glioblastoma or malignant glioma                                            |
| AMG-673       | CD3 \times CD33  | NCT03249819      | Early Phase 1 | Acute myeloid leukemia                                                        |
| AMG-757       | CD3 \times DLL3  | NCT03319940      | Phase 1  | Small cell lung carcinoma                                                   |
| AMV-564, TandAb T564 | CD3 \times CD33 | NCT03144245      | Phase 1  | Acute myeloid leukemia                                                   |
| AMV-673       | CD3 \times CD123 | NCT03647800      | Phase 1  | Acute myeloid leukemia, myelodysplastic syndrome                            |
| BI-836909, AMG-420 | CD3 \times BCMA | NCT02514239      | Phase 1  | Multiple myeloma                                                             |
| EM-901, CC-93269 | CD3 \times BCMA  | NCT03486067      | Phase 1  | Multiple myeloma                                                             |
| ERY-974       | CD3 \times GPC3  | NCT02748837      | Phase 1  | Solid tumors                                                                 |
| GBR-1302      | CD3 \times HER2  | NCT02829172      | Phase 1  | Her2 positive solid tumors                                                   |
| GBR-1342      | CD3 \times CD38  | NCT03309111      | Phase 1/2 | Breast cancer                                                                |
| GEM-333       | CD3 \times CD33  | NCT03516760      | Phase 1  | Acute myeloid leukemia                                                       |
| GEM-3PSCA, GEM3PSCA | CD3 \times PSMA  | NCT03927573      | Phase 1  | Non-small cell lung cancer, breast cancer, pancreatic cancer, urogenital cancer |
| IGM-2323      | CD3 \times CD20  | NCT04082936      | Phase 1  | Non-Hodgkin lymphoma, follicular lymphoma, mantle cell lymphoma, marginal zone lymphoma |
| JNJ-67571244, JNJ-1244 | CD3 \times CD33 | NCT03915379      | Phase 1  | Leukemia                                                                     |
| JNJ-63709178, JNJ-9178 | CD3 \times CD123 | NCT02715011      | Phase 1  | Leukemia                                                                     |
| JNJ-64007957, JNJ-7957 | CD3 \times BCMA | NCT03145181      | Phase 1  | Hematological malignancies                                                  |
| JNJ-63898081, JNJ-8081 | CD3 \times PSMA | NCT03926013      | Phase 1  | Neoplasms                                                                   |
| Orlotamab, MGD-009 | CD3 \times B7-H3 | NCT03406949      | Phase 1  | Advanced solid tumors                                                       |
| Pasotuxizumab, AMG-212, (BAY2010112/MT112) | CD3 \times PSMA | NCT01723475      | Phase 1  | Prostatic neoplasms                                                        |
| PF-06671008   | CD3 \times CDH3  | NCT02659631      | Phase 1  | Neoplasms                                                                   |
| PF-06863135, PF-3135 | CD3 \times BCMA | NCT03269136      | Phase 1  | Multiple myeloma                                                             |
| REGN-5458     | CD3 \times BCMA  | NCT03761108      | Phase 1/2 | Multiple myeloma                                                             |
| RG-6194, BTRC-4017A | CD3 \times HER2 | NCT03448042      | Phase 1  | Solid tumors                                                                 |
| TNB-383B      | CD3 \times BCMA  | NCT03933735      | Phase 1  | Multiple myeloma                                                             |
| XmAb-13676, THG-338 | CD3 \times CD20 | NCT02924402      | Phase 1  | B cell non-Hodgkin lymphoma                                                  |
| XmAb-14045, SQZ-622 | CD3 \times CD123 | NCT02730312      | Phase 1  | Acute myelogenous leukemia, B cell acute lymphoblastic leukemia, blastic plasmacytoid dendritic cell neoplasm, chronic myeloid leukemia |
FDA approval [114–117]. Clinical trials are also ongoing, and CAR T cells specific for CD30 (CD30-targeting CARs) have shown potential to treat Hodgkin’s lymphoma (HL) in two phase 1/2 clinical trials (NCT02690545, NCT02917083) [118]. A clinical trial of anti-CD7 universal CAR-T (U-CAR-T) cells indicated that patients with T cell lymphoma displayed robust CAR-T cell expansion (NCT04264078) [119]. In a phase 1/2 clinical study (NCT01869166), anti-EGFR CAR-T cells were found to be a feasible therapeutic strategy for EGFR-positive patients with NSCLC [120].

However, the success of CAR T cell therapy is yet to be applied clinically. Several impediments have been encountered, namely, poor availability of tumor specific antigens, immunosuppressive characteristics of the TME, and variability in manufacturing quality and high processing costs [121–123]. The use of “off-the-shelf” allogeneic CAR T cells from healthy donors could potentially overcome these issues. Allogeneic T cells are primarily derived from peripheral blood mononuclear cells, embryonic stem cells, and induced pluripotent stem cells. Allogeneic CAR T products can markedly decrease the costs owing to industrialized and scaled-up production, thereby rendering CAR T treatment immediately accessible to a large number of patients due to the batch manufacturing of cryopreserved T cells. The use of allogeneic cells would also provide a high-quality product based on donor selection and allow for standardized dosing and re-dosing and a combination of CAR targets [122,123]. Other major issues must be addressed, including toxicities such as graft versus host disease (GVHD) and limited anti-tumor efficacy against solid tumors. Various safeguarding strategies, such as applying non-αβ T cells including γδ T cells [124], gene editing with αβ T cell receptor (TCR) deletion [125], and using virus-specific T cells [126] or donor-derived allogeneic T cells [127], are needed to improve the clinical safety of CAR T cell therapy. All these techniques have been designed to specifically reduce GVHD toxicity.

Although CAR T cell therapies have shown unsatisfactory efficacy in solid tumors, many promising methods can be applied for optimization. Improving CAR T structures [128] and combining with different treatment strategies such as chemotherapy [129], local therapy [130], checkpoint blockades [131], bsAbs [132], epigenetic modulators [133], vaccines [134], and oncolytic viruses [135] have all been explored to enhance the persistence and antitumor activity of CAR T cell therapy.

Despite the bumpy road ahead, the future of CAR T cell therapy looks promising because of the continuous evolution of advanced gene editing techniques and novel solutions. These innovations will help “off-the-shelf”
allogeneic CAR T cell therapy to be effective, safe, and perhaps even revolutionize cancer treatment.

**Therapeutic cancer vaccines**

Cancer vaccines trigger immune responses against tumor cells by amplifying and broadening antigen-specific T cells [136]. Tumor antigens, immune adjuvants, delivery vehicles, and formulations are the four key components of therapeutic vaccines and are vital for efficacy. Tumor antigens can be delivered in the form of genetic vaccines (DNA/RNA/viral), protein/peptide vaccines, and cell vaccines. Delivery method is also a major factor influencing vaccine efficacy [136].

Antigens for tumor vaccines include TAAs and tumor-specific antigens (TSAs). Early cancer vaccines focused on TAAs, self-antigens that have elevated levels on tumor cells but may also be expressed on normal cells. However, TAAs lacking tumor specificity increase the risk of autoimmune toxicities and have been unsuccessful in generating effective antitumor immune responses due to immune tolerance [136,137]. TSAs comprise antigens expressed by neoantigens or oncoviruses and are found exclusively in cancer cells. Neoantigen-based cancer vaccines are tumor-specific, can enhance a tumor-specific T cell response, and prevent toxicities caused by “off-target” damage. Recent development on bioinformatics technologies has enabled the systematic identification of tumor neoantigens; several promising studies have explored neoantigen cancer vaccines [138]. In a phase 1 clinical trial, Ott et al. reported a neoantigen vaccine that was formulated with up to 20 personized HLA-A/B-restricted peptides and has expanded neoantigen-specific T cells in patients with melanoma (NCT01970358) [139]. After a 4-year median follow-up of neoantigen vaccine therapy, a persistent T cell response was observed in patients with melanoma [140]. Neoantigen-specific T cells from peripheral blood also show the potential to migrate into intracranial tumors in glioblastoma after surgical resection cases in a phase 1b clinical trial (NCT02287428) [141]. These initial studies suggest that neoantigen-specific cancer vaccines are safe in patients with melanoma and glioblastoma. For further understanding on their therapeutic efficacy, in-depth studies must be conducted on the function of vaccine-induced T cells and the persistence of neoantigen-specific memory T cells.

Most therapeutic cancer vaccines are in ongoing trials, and their development can possibly enhance the efficacy of immunotherapy. In a phase 1b study of a neoantigen-based peptide vaccine NEO-PV-01 in combination with a PD-1 inhibitor, epitope spreading was detected post-vaccination and correlated with improved progression free survival in patients (NCT02897765) [142]. Compared with sunitinib monotherapy, sunitinib in combination with ilixadencel, a cell-based allogeneic off-the-shelf product, exhibited a higher overall response rate in patients with synchronous metastatic renal cell carcinoma [143]. In a clinical trial of personalized tumor lysate-pulsed DCs for patients with recurrent ovarian cancer, a vaccine plus therapy seemed to improve the overall survival compared with a low-dose cyclophosphamide and bevacizumab combination therapy [144].

Although the above preliminary findings are encouraging, numerous challenges remain to be addressed. First, further discovery of personalized neoantigen targets is required to maximize their effects. Second, delivery strategies are an important factor affecting vaccine efficacy; the effectiveness of different delivery methods varies among tumor types. Finally, when a vaccine is being combined with existing treatment approaches, the timing, sequence, and dose of combination therapy must be further explored.

**Challenges and future direction**

New immuno-therapeutic approaches provide opportunities for further drug development and bring benefits to patients. However, challenges persist during their development. Therefore, further basic and clinical research is needed.

**Assessment of combination therapy**

Given that anti-tumor immunity involves various steps, rational combinations to modulate different biological steps might strengthen anti-tumor responses. Effective transformation from basic discovery to clinical application could be achieved by exploring the molecular mechanisms and optimizing the strategies and timing of combination therapy to maximize its effects. The combination of four components (anti-PD1 therapy, tumor antigen-targeting antibody, interleukin-2, and a T cell vaccine) that engage in innate and adaptive immune responses was reported to eliminate large tumors in mouse model [145]. However, most drugs are in the early stages of clinical trials with complicated combinations and pose various challenges, specifically how to maximize their synergistic effects and how to avoid combinational toxicities. For a partial solution, MORPHEUS and FRACTION platforms were designed to evaluate the safety and effectiveness of combination immunotherapies in multiple phase 1b/2 trials [146,147]. A novel Quick efficacy seeking trial (QuEST1) was also designed to assess different immunotherapy combinations in patients with prostate cancer [148]. The rational selection of the combination and dosage based on known molecular mechanisms to maximize their synergistic effects is yet to be elucidated.
Validated biomarkers

Over 3000 interventional clinical trials of immunological drugs either alone or in combination are being conducted globally [149]. Nevertheless, the clinical benefits of many novel immunotherapies cannot be determined at this stage. Strategies for the identification of valid biomarkers are essential in identifying patients who will benefit the most. Many current clinical trials include the detection of serial sampling of peripheral blood or tumor specimens for the analyses of corresponding biomarkers (such as NCT01928576 and NCT03220477). In a phase 2 study of immunotherapy combinations of motolimod and doxorubicin for ovarian cancer, statistically significant differences in the overall survival of motolimod-treated patients were observed in a subgroup of patients who experienced injection site reactions; this investigation may provide biomarkers to evaluate the efficacy of combinational immunotherapies [150]. Owing to the complex interactions required for effective treatment, the development of actionable information and identifying feasible markers that can accurately classify patients is imperative.

Autoimmune toxicities

The mechanisms of immune-related toxicities must be understood to produce the best personalized treatment approach. Small changes in the molecular structures of small molecules may lead to tremendous variations in efficacy and toxicity. Diverse challenges have emerged during the exploration of bsAbs, such as reducing toxicity and immunogenicity induced by neo-epitopes, satisfying thresholds for sensitizing various molecular pathways, and assuring the quantity and quality of bsAbs.

The application of CAR T is also not without concerns. This treatment can lead to adverse effects, such as cytokine release syndrome and on-target off-tumor toxicity. Early recognition of cytokine release syndrome and aggressive steroid administration in CAR T treatment are important [151]. Moreover, drug–drug interactions must be considered for the toxicities of combination treatments. In a phase 2 study, the combination of pembrolizumab plus oral azacitidine CC-486 was associated with an increase in treatment-related adverse events compared with the pembrolizumab plus placebo group. This phenomenon can be attributed to the intestinal and hematological toxicities noted for the oral formulation of azacitidine [152].

Improving manufacturing practices

The production of bsAbs, CAR T cells, and neoantigen-based vaccines is expensive and time consuming. In the development of biological products, optimizing the structures and workflows according to the biological mechanisms requires special attention. Designing a reasonable antibody structure according to different effect mechanisms is the focus of current bsAb research and development.

Complete CAR T cell therapy is complex compared with autologous products; however, allogeneic CAR T products offer the advantages of industrialized production and low costs [122]. Manufacturing some biological products is also time consuming, and the production of personalized vaccines is more expensive than off-the-shelf therapeutic agents. Vaccine preparation usually takes 3–5 months at best [139]. Technological developments, such as automated flow peptide production, might help promote peptide manufacturing and decrease the production time of personalized vaccines [153]. For these emerging immunotherapy drugs, their research, development, and production time and costs must be considered.

Conclusions

Immune checkpoint therapies, such as PD-1, PD-L1, and CTLA-4 antibodies, have made considerable headway in tumor treatments for the past decade. However, only a small number of cases respond to immunotherapy and are often accompanied by adverse reactions. Therefore, new treatment options are essential to enhance immunotherapy efficacy, overcome immunosuppression, and reduce toxicity. Understanding novel immuno-oncology therapeutic strategies allow us to provide additional opportunities for patients with advanced cancer. Small molecule drugs, bsAbs, CAR-T treatment, and cancer vaccines provide appealing avenues for immunotherapy. Related preliminary preclinical and clinical studies are already underway. Cost of treatment, lack of biomarker responses, and combination therapies targeting different immune mechanisms remain as challenges to be overcome. Nevertheless, these emerging strategies can bring about new opportunities for patients with cancer.

Compliance with ethics guidelines

Hongyun Zhao, Fan Luo, Jinhui Xue, Su Li, and Rui-Hua Xu declare that they have no conflicts of interest. This manuscript is a review article and does not involve a research protocol requiring approval from relevant institutional review board or ethics committee.

Electronic Supplementary Material Supplementary material is available in the online version of this article at https://doi.org/10.1007/s11684-021-0886-x and is accessible for authorized users.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format,
as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder.

To view a copy of this license, visit https://creativecommons.org/licenses/by/4.0/.

References
1. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, Powderly JD, Carvajal RD, Sosman JA, Atkins MB, Leming PD, Spigel DR, Antonia SJ, Horn L, Drake CG, Pardoll DM, Chen L, Sharifman WH, Anders RA, Taube JM, McMiller TL, Xu H, Korman AJ, Jure-Kunkel M, Agrawal S, McDonald D, Kollias GD, Gupta A, Wigginton JM, Szol M. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med 2012; 366(26): 2443–2454
2. Skoulidis F, Goldberg ME, Greenawalt DM, Hellmann MD, Awad MM, Gainor JF, Schroock AB, Hartmaier RJ, Trabucco SE, Gay L, Ali SM, Elvin JA, Singal G, Ross JS, Fabrizio D, Szabo PM, Chang H, Sasson A, Sriivasan S, Kimov S, Szustakowski J, Vitaska P, Edwards R, Buffil JA, Sharma N, Ou SI, Peled N, Spigel DR, Rizvi H, Aguilar EJ, Carter BW, Erasmus J, Halpenny DF, McDermott DF, Long NM, Nishino M, Galan-Cobo A, Hamdi H, Hirz T, Tong P, Wang J, Rodríguez-Canales J, Villalobos PA, Parra ER, Kalhor N, Soll LM, Sauter JL, Jungbluth AA, Mino-Kenudson M, Azimiz R, Elamin YY, Zhang J, Leonard GC, Jiang F, Wong KK, Lee JJ, Papadimitrakopoulou VA, Westuba II, Miller VA, Frampton GM, Wolchok JD, Shaw AT, Jänne PA, Stephens PJ, Rudin CM, Geese WJ, Albacker LA, Heymach JV. STK11/LKB1 mutations and PD-1 inhibitor resistance in KRAS-mutant lung adenocarcinoma. Cancer Discov 2018; 8(7): 822–835
3. Postow MA, Sidlow R, Hellmann MD. Immune-related adverse events associated with immune checkpoint blockade. N Engl J Med 2018; 378(2): 158–168
4. van der Zanden SY, Luimstra JJ, Neefjes J, Borst J, Ovaa H. STING and innate immune recognition of immunogenic tumors. Immunity 2014; 41(5): 373–384
5. Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. Nat Immunol 2010; 11(5): 373–384
6. Work Group; Invited Reviewers, Kim JYS, Kozlov JH, Mittal B, Moyer J, Olencki T, Rodgers P. Guidelines of care for the management of basal cell carcinoma. J Am Acad Dermatol 2018; 78(3): 540–559
7. Donin NM, Chamie K, Lenis AT, Pantuck AJ, Reddy M, Kivlin DK, Halldicck J, Pozzi R, Hakim G, Lamm DL, Belkoff LH, Belldegrun AS, Holden S, Shore N. A phase 2 study of TMX-101, intravesical imiquimod, for the treatment of carcinoma in situ bladder cancer. Urol Oncol 2017; 35(2): 39.e1–39.e7
8. Dietsch GN, Lu H, Yang Y, Morishima C, Chow LQ, Disis ML, Hershberg RM. Coordinated activation of Toll-like receptor 8 (TLR8) and NLRP3 by the TLR8 agonist, VTX-2337, ignites macrophages to enhance cancer immunotherapy. Nat Biomed Eng 2018; 2(8): 578–588
9. Corrales L, Glickman LH, McWhirter SM, Kanne DB, Sivick KE,
Katibah GE, Woo SR, Lemmens E, Banda T, Leong JJ, Metchette K, Dubensky TW Jr, Gajewski TF. Direct activation of STING in the tumor microenvironment leads to potent and systemic tumor regression and immunity. Cell Rep 2015; 11(7): 1018–1030.

Sivick KE, Desbien AL, Glickman LH, Reiner GL, Corrales L, Suri NH, Hudson TE, Vu UT, Francica BJ, Banda T, Katibah GE, Kanne DB, Leong JJ, Metchette K, Bruml JR, Nubukuro CO, McKenna JM, Feng Y, Zheng L, Bender SL, Cho CY, Leong ML, van Elsas A, Dubensky TW Jr, McWhirter SM. Magnitude of therapeutic STING activation determines CD8 T cell-mediated anti-tumor immunity. Cell Rep 2018; 25(11): 3074–3085.e5

Meric-Bernstam F, Sandhu S K, Hamid O, Spreamico A, Kasper S, Dummer R, Shimizu T, Steeghs N, Lewis N, Talluto C. Phase Ib study of MIW815 (ADU-S100) in combination with spartalizumab (PDR001) in patients (pts) with advanced/metastatic solid tumors or lymphomas. J Clin Oncol 2019; 37 (15_suppl): 2507

Tye H, Kennedy CL, Najdovska M, McLeod L, McCormack W, Hughes N, Dev A, Siavert W, Ooi CH, Ishikawa TO, Oshima H, Bhatral BS, Parker AE, Oshima M, Tan P, Jenkins BJ. STAT3-driven upregulation of TLR2 promotes gastric tumorigenesis independent of tumor inflammation. Cancer Cell 2012; 22(4): 466–478

Ochi A, Graffio CS, Zambriniris CP, Rehaman H, Hackman M, Fallon N, Barilla RM, Henning JR, Jamal M, Rao R, Greco S, Deutsch M, Medina-Zea MV, Bin Saeed U, Ego-Osuala MO, Hujdu C, Miller G. Toll-like receptor 7 regulates pancreatic carcinogenesis in mice and humans. J Clin Invest 2012; 122(11): 4118–4129

Li X, Wenes M, Romero P, Huang SC, Fendt SM, Ho PC. Navigating metabolic pathways to enhance antitumour immunity and immunotherapy. Nat Rev Clin Oncol 2019; 16(7): 425–441

Liu X, Shin N, Koblish HK, Yang G, Wang Q, Wang K, Leffert L, Hanksy MJ, Thomas B, Rupar M, Waeltz P, Bowman KJ, Polam P, Sparks RB, Yue EW, Li Y, Wynn R, Fridman JS, Burn TC, Combs AP, Newton RC, Scherle PA. Selective inhibition of IDO1 effectively regulates mediators of antitumor immunity. Blood 2010; 115(17): 3520–3530

Luke J, Taberner J, Joshua A, Desai J, Varga A, Moreno V, Gomez-Roca C, Markman B, Braud F, Patel S, Carlino M, Sui L, Curigliano G, Liu Z, Ishii Y, Wind-Rotolo M, Basciano P, Azrilevich A, Gelmon K. BMS-986205, an indoleamine 2, 3-dioxygenase 1 inhibitor (IDO1i), in combination with nivolumab (atezolizumab): updated safety across all tumor cohorts and efficacy in advanced bladder cancer (advBC). J Clin Oncol 2019; 37(7 suppl): 3220–3228

Long GV, Dummer R, Hamid O, Gajewski TF, Caglevie C, Dalle S, Arance A, Carlino MS, Grob JJ, Kim TM, Demidov L, Robert C, Larkin J, Anderson JR, Maleski J, Jones M, Diede SJ, Mitchell TC. Epacadostat plus pembrolizumab versus placebo plus pembrolizumab in patients with unresectable or metastatic melanoma (ECHO-301/KEYNOTE-252): a phase 3, randomised, double-blind study. Lancet Oncol 2019; 20(8): 1083–1097.

Adams JL, Smothers J, Srinivasan R, Hoos A. Big opportunities for small molecules in immuno-oncology. Nat Rev Drug Discov 2015; 14(9): 603–622

Steggerda SM, Bennett MK, Chen I, Emberley E, Huang T, Janes JR, Li W, MacKinnon AL, Makkouk A, Marguier G, Murray PJ, Neou S, Pan A, Parlati F, Rodriguez MLM, Van de Velde LA, Wang T, Works M, Zhang J, Zhang W, Gross MI. Inhibition of arginase by CB-1158 blocks myeloid cell-mediated immune suppression in the tumor microenvironment. J Immunother Cancer 2017; 5(1): 101

Johnson M O, Wolf M, Madden M Z, Andrejeva G, Sugii A, Contreras DC, Maseda D, Liberti M V, Paz K, Kishon R J, Johnson M E, de Cubas A, Wu P, Li G, Zhang Y, Newcomb D C, Wells A D, Restifo N P, Rathmell W K, Locasale J W, Davila M L, Blazar BR, Rathmell JC. Distinct regulation of TH17 and TH1 cell differentiation by glutaminase-dependent metabolism. Cell 2018; 175(7): 1780–1795.e1719

Deaglio S, Dwyer KM, Gao W, Friedman D, Usheva A, Eron J, Chen JF, Enyjoji K, Linden J, Oukka M, Kuchroo VK, Strom TB, Robson SC. Adenosine generation catalyzed by CD39 and CD73 expressed on regulatory T cells mediates immune suppression. J Exp Med 2007; 204(6): 1257–1265

Beavis PA, Milenkovski N, Henderson MA, John LB, Allard B, Loi S, Kershaw MH, Stagg J, Darcy PK. Adenosine receptor A2A blockade increases the efficacy of anti-PD-1 through enhanced antitumor T-cell responses. Cancer Res 2015; 75(3): 506–517

Emens L, Powderly J, Fong L, Brody J, Forde P, Hellmann M, Hughes B, Kommur S, Loi S, Luke J C. CPI-444, an oral adenosine A2a receptor (A2aR) antagonist, demonstrates clinical activity in patients with advanced solid tumors. Cancer Res 2017; 77(13 suppl): CT119

Ivanov II, McKenzie BS, Zhou L, Tadokoro CE, Lepelley A, Lafaille JJ, Cua DJ, Littman DR. The orphan nuclear receptor RORα directs the differentiation program of proinflammatory IL-17+ T helper cells. Cell 2006; 126(6): 1121–1133

Hu X, Liu X, Moisan J, Yang W, Lesch CA, Spooner C, Morgan RW, Zawidzka EM, Mertz D, Bousley D, Majchrzak K, Kryczek I, Taylor C, Van Huis C, Salitizky D, Hurd A, Achier TD, Toogood PL, Glick GD, Paulos CM, Zou W, Carter LL. Synthetic RORγt agonists regulate multiple pathways to enhance antitumor immunity. OncolImmunology 2016; 5(12): e1254854

Herbert S, Sawyer JS, Stauber AJ, Gucgortei A, Driscoll KE, Estrem ST, Cleverly AL, Desai A, Guba SC, Benhadji KA, Slapak CA, Lahn MM. Clinical development of galunisertib (LY2157299 monohydrate), a small molecule inhibitor of the tumor microenvironment leading to durable, complete responses, as monotherapy and in combination with checkpoint blockade. J Immunother Cancer
39. Yokosuka T, Takamatsu M, Kobayashi-Imanishi W, Hashimoto-Tane A, Azuma M, Saito T. Programmed cell death 1 forms negative costimulatory microclusters that directly inhibit T cell receptor signaling by recruiting phosphate SHP2. J Exp Med 2012; 209(6): 1201–1217

40. Zhao M, Guo W, Wu Y, Yang C, Zhong L, Deng G, Zhu Y, Liu W, Gu Y, Lu Y, Kong L, Meng X, Xu Q, Sun Y. SHP2 inhibition triggers anti-tumor immunity and synergizes with PD-1 blockade. Acta Pharm Sin B 2019; 9(2): 304–315

41. Dammeijer F, Lievense LA, Kajien-Lambers ME, van Nimwegen M, Bezemker K, Hegmans JP, van Hall T, Hendriks RW, Aerts JG. Depletion of tumor-associated macrophages with a CSF-1R kinase inhibitor enhances antitumor immunity and survival induced by DC immunotherapy. Cancer Immunol Res 2017; 5(7): 535–546

42. Gumbleton M, Sudan F, Engelman RW, Russo CM, Glenadel Q, Tibbitts T, Rowley AM, DiNitto JP, Brophy EE, Hladnik LM, Kulkarni S, Abboud CN, Cashen AF, Stockerl-Goldstein KE, Vij R, Westervelt P, DiPersio JF. A phase I/2 study of chemosensitization with the CXCR4 antagonist plerixafor in relapsed or refractory acute myeloid leukemia. Blood 2012; 119(17): 3917–3924

43. Evans CA, Liu T, Lescarbeau A, Nair SJ, Grenier L, Pradeilles JA, Gumbleton M, Sudan R, Fernandes S, Engelman RW, Russo CM, Chen Y, Ramjiawan RR, Reiberger T, Ng MR, Hato T, Huang Y, Zlotnik A, Yoshie O. The chemokine superfamily revisited. Immunity 2012; 7(9): 862–867

44. Yokosuka T, Esaki K, Tachibana T, Ishii S, Soeda T, Muto A, Kawabe Y, Igawa T, Tsunoda H, Mogami K, Shimada H, Hattori K. Factor VIII-like mimetic cofactor activity of a bispecific antibody to factors IXa/IXa and Xa/Xa, emicizumab, depends on its ability to bridge the antigens. Thromb Haemost 2017; 117(7): 1348–1357

45. Dickopf S, Georges GJ, Brinkmann U. Format and geometries improve T cell function by pulsatile inhibition of SHIP1 improves antitumor immunity and survival. Sci Signal 2017; 10(500): eaam5535

46. Evans CA, Liu T, Lescarbeau A, Nair SJ, Grenier L, Pradeilles JA, Gumbleton M, Sudan R, Fernandes S, Engelman RW, Russo CM, Chen Y, Ramjiawan RR, Reiberger T, Ng MR, Hato T, Huang Y, Zlotnik A, Yoshie O. The chemokine superfamily revisited. Immunity 2012; 7(9): 862–867

47. Yokosuka T, Esaki K, Tachibana T, Ishii S, Soeda T, Muto A, Kawabe Y, Igawa T, Tsunoda H, Mogami K, Shimada H, Hattori K. Factor VIII-like mimetic cofactor activity of a bispecific antibody to factors IXa/IXa and Xa/Xa, emicizumab, depends on its ability to bridge the antigens. Thromb Haemost 2017; 117(7): 1348–1357

48. Dickopf S, Georges GJ, Brinkmann U. Format and geometries improve T cell function by pulsatile inhibition of SHIP1 improves antitumor immunity and survival. Sci Signal 2017; 10(500): eaam5535

49. Evans CA, Liu T, Lescarbeau A, Nair SJ, Grenier L, Pradeilles JA, Gumbleton M, Sudan R, Fernandes S, Engelman RW, Russo CM, Chen Y, Ramjiawan RR, Reiberger T, Ng MR, Hato T, Huang Y, Zlotnik A, Yoshie O. The chemokine superfamily revisited. Immunity 2012; 7(9): 862–867

50. Nisonoff A, Wissler FC, Lipman LN. Properties of the major component of a peptic digest of rabbit antibody. Science 1960; 132(3441): 1770–1771

51. Labrijn AF, Jannaat ML, Reichert JM, Parren PWH. Bispecific antibodies: a mechanistic review of the pipeline. Nat Rev Drug Discov 2019; 18(8): 585–608

52. Chelius D, Ruf P, Gruber P, Plösch M, Liedtke R, Gansberger E, Hess W, Vasiliu M, Lindhofer H. Structural and functional characterization of the trifunctional antibody catumaxomab. MAbs 2010; 2(3): 309–319

53. Klinger M, Brandl C, Zugmaier G, Hijazi Y, Bargou RC, Topp MS, Gökbuget N, Neumann S, Goebeler M, Viardot A, Stelljes M, Brüggemann M, Hoelzer D, Gansberger E, Hofner D, Nogorski D, Baeuerle PA, Wolf A, Kufer P. Immunopharmacologic response of patients with B-lineage acute lymphoblastic leukemia to continuous infusion of T cell-engaging CD19/CD3-bispecific BiTE antibody blinatumomab. Blood 2012; 119(26): 6226–6233

54. Kitazawa T, Esaki K, Tachibana T, Ishii S, Soeda T, Muto A, Kawabe Y, Igawa T, Tsunoda H, Mogami K, Shimada H, Hattori K. Factor VIII-like mimetic cofactor activity of a bispecific antibody to factors IXa/IXa and Xa/Xa, emicizumab, depends on its ability to bridge the antigens. Thromb Haemost 2017; 117(7): 1348–1357

55. Dickopf S, Georges GJ, Brinkmann U. Format and geometries improve T cell function by pulsatile inhibition of SHIP1 improves antitumor immunity and survival. Sci Signal 2017; 10(500): eaam5535
bodies. MAbs 2012; 4(2): 182–197
65. Nie S, Wang Z, Moscoso-Castro M, D’Souza P, Lei C, Xu J, Gu J. Biology drives the discovery of bispecific antibodies as innovative therapeutic agents. Antib Ther 2020; 3(1): 18–62
66. Grugan KD, Dom K, Jarantow SW, Bushey BS, Pardinas JR, Laquerre S, Moores SL, Chiu ML. Fc-mediated activity of EGFR x c-Met bispecific antibody JNJ-61186372 enhanced killing of lung cancer cells. MAbs 2019; 9(1): 114–126
67. Wang Q, Chung CY, Chough S, Betenbaugh MJ. Antibody glycoengineering strategies in mammalian cells. Biotechnol Bioeng 2018; 115(6): 1378–1393
68. Kontermann RE, Brinkmann U. Bispecific antibodies. Drug Discov Today 2015; 20(7): 838–847
69. Gupta A, Kumar Y. Bispecific antibodies: a novel approach for targeting prominent biomarkers. Hum Vaccin Immunother 2020; 16(11): 2831–2839
70. Kontermann RE, Brinkmann U. Bispecific antibodies. Drug Discov Today 2015; 20(7): 838–847
71. Xu T, Tao X, Wang X, Li Q, Minjie P, Zhang H, Han L, Zhang Q. Patent US 80808043 (B2); PCT/CN2016/070447. 2020
72. Li F, Zhang B, Ye P, Zhao J, Huang S, Jin C. Patent US 9745382 B1; PCT/CN2017/093816, 2017
73. Liu J, Song N, Yang Y. Patent WO2018177324 (A1); PCT/CN2018/080588. 2018
74. Liu J, Song N, Yang Y. Patent WO2018090950 (A1); PCT/CN2016/092679. 2017
75. Xu T, Dong Y, Wang P. Patent US 20180291103 (A1); PCT/CN2016/092679. 2017
76. Wu C. Patent US 10266608 (B2); PCT/US2014/072336. 2019
77. Eckelman B, Timmer JC, Hata C, Jones KS, Hussain A, Razai AS, Wu C. Patent US 10266608 (B2); PCT/US2014/072336. 2019
78. Xu T, Tau X, Wang X, Li Q, Minjie P, Zhang H, Han L, Zhang Q. Patent US 10808043 (B2); PCT/CN2016/070447. 2020
79. Li F, Zhang B, Ye P, Zhao J, Huang S, Jin C. Patent US 9745382 (B1); PCT/CN2017/093816, 2017
80. Kontermann RE, Brinkmann U. Bispecific antibodies. Drug Discov Today 2015; 20(7): 838–847
81. Kontermann RE, Brinkmann U. Bispecific antibodies. Drug Discov Today 2015; 20(7): 838–847
82. Kontermann RE, Brinkmann U. Bispecific antibodies. Drug Discov Today 2015; 20(7): 838–847
83. Tian W, Li S. Patent WO2018166650; PCT/CN2018/079187. 2018
84. Huang Y, Zhang F, Xi G. Patent WO2019109357; PCT/CN2017/115323. 2019
85. Hinner MJ, Aiba RSB, Wiedenmann A, Schlosser C, Allersdorfer A, Matschiner G, Rothe C, Moebius U, Kohrt HE, Ohlwill SA. Costimulatory T cell engagement via a novel bispecific anti-CD137/anti-HER2 protein. J Immunother Cancer 2015; 3(Suppl 2): 187
86. Chames P, Baty D. Bispecific antibodies for cancer therapy. Curr Opin Drug Discov Devel 2009; 12(2): 276–283
87. Poole RM. Pembrolizumab: first global approval. Drugs 2014; 74 (16): 1973–1981
88. Markham A. Atezolizumab: first global approval. Drugs 2016; 76 (12): 1227–1232
89. Kim ES. Avelumab: first global approval. Drugs 2017; 77(8): 929–937
90. Syed YY. Durvalumab: first global approval. Drugs 2017; 77(12): 1369–1376
91. Osipov A, Zaidi N, Laheru DA. Dual checkpoint inhibition in pancreatic cancer: revealing the limitations of synergy and the potential of novel combinations. JAMA Oncol 2019; 5(10): 1438–1439
92. Beck M, Borchgrev K, O’Byrne KJ. Nivolumab plus ipilimumab in non-small-cell lung cancer. Future Oncol 2019; 15(19): 2287–2302
93. Winer A, Ghatapal A, Bubens N, Anari F, Varshavsky A, Kasireddy V, Liu Y, El-Deiry WS. Dual checkpoint inhibition with ipilimumab plus nivolumab after progression on sequential PD-1/PDL-1 inhibitors pembrolizumab and atezolizumab in a patient with Lynch syndrome, metastatic colon, and localized urothelial cancer. Oncologist 2019; 24(11): 1416–1419
94. Hassel JC, Heinzerling L, Aberle J, Bühr O, Eigentler TK, Grimm MO, Grünwald V, Leipe J, Reinmuth N, Tietze JK, Trojan J, Zimmer L, Gutzmer R. Combined immune checkpoint blockade (anti-PD-1/anti-CTLA-4): evaluation and management of adverse drug reactions. Cancer Treat Rev 2017; 57: 36–49
95. Topalian SL, Drake CG, Pardoll DM. Targeting the PD-1/B7-H1 (PD-L1) pathway to activate anti-tumor immunity. Curr Opin Immunol 2012; 24(2): 207–212
96. Hugo W, Zaretzky JM, Sun L, Song C, Moreno BH, Hu-Lieskovsk S, Berent-Maoz B, Pang J, Chmielowski B, Cherry G, Seja E, Lomeli S, Kong X, Kelley MC, Sosman JA, Johnson DB, Ribas A, Lo RS. Genomic and transcriptomic features of response to anti-PD-1 therapy in metastatic melanoma. Cell 2016; 165(1): 35–44
97. Engelman JA, Zejnilullah K, Mitsudomi T, Song Y, Hyland C, Park JO, Lindeman N, Gale CM, Zhao X, Christensen J, Kosaka T, Holmes AJ, Rogers AM, Cappuzzo F, Mok T, Lee C, Johnson BE, Cantley LC, Jänne PA. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. Science 2007; 316(5827): 1039–1043
98. Turke AB, Zejnilullah K, Wu YL, Song Y, Dias-Santagata D, Lifshits E, Toschi L, Rogers A, Mok T, Sequist L, Lindeman NI, Murphy C, Akhavanfard S, Yeap BY, Xiao Y, Capelletti M, Iafrate AJ, Lee C, Christensen JG, Engelman JA, Jänne PA. Preexistence and clonal selection of MET amplification in EGFR mutant NSCLC. Cancer Cell 2010; 17(1): 77–88
99. Yano S, Yamada T, Takeuchi S, Tachibana K, Minami Y, Yatabe Y, Mitsudomi T, Tanaka H, Kimura T, Kudoh S, Nokihara H, Ohe Y, Yokota J, Uramoto H, Yasumoto K, Kiura K, Higashiyama M, Oda M, Saito H, Yoshida J, Kondoh K, Noguchi M. Hepatocyte growth factor expression in EGFR mutant lung cancer with intrinsic and acquired resistance to tyrosine kinase inhibitors in a Japanese cohort. J Thorac Oncol 2011; 6(12): 2011–2017
100. van Lengerich B, Agnew C, Puchner EM, Huang B, Jura N. EGF and NRG induce phosphorylation of HER3/ERBB3 by EGFR using distinct oligomeric mechanisms. Proc Natl Acad Sci USA
101. Mujoo K, Choi BK, Huang Z, Zhang N, An Z. Regulation of ERBB3/HER3 signaling in cancer. Oncotarget 2014; 5(21): 10222–10236.

102. Tian W, Li S, Chen D, Liang G, Zhang L, Zhang W, Tu X, Peng L, Weng J, Zhao G. Preclinical development of a bispecific antibody-trap selectively targeting CD47 and CD40 for the treatment of B cell lineage cancer. Cancer Res 2019; 79(13 Suppl): Abstract nr 545.

103. Robert B, Dorvillius M, Buchegger F, Garambois V, Mani JC, Puginères M, Mach JP, Pélégrin A. Tumor targeting with newly designed biparatopic antibodies directed against two different epitopes of the carcinoembryonic antigen (CEA). Int J Cancer 1999; 81(2): 285–291.

104. Wei H, Cai H, Jin Y, Wang P, Zhang Q, Lin Y, Wang W, Cheng J, Zeng N, Xu T, Zhou A. Structural basis of a novel heterodimeric Fc for bispecific antibody production. Oncotarget 2017; 8(31): 51037–51049.

105. Li F, Zhang B, Ye P, Zhao J, Huang S, Jin C. Bispecific anti-HER2 antibody. Patent US 9745382 (B1); PCT/CN2017/093816. 2017.

106. Center for Drug Evaluation of the National Medical Products Authority. http://www.cde.org.cn/ (accessed August 31, 2020).

107. Li B, Xia Y, Wang Z M, Zhang P. Patent MX2019002254 (A). 2019.

108. Du X, Liu M, Su J, Zhang P, Tang F, Ye P, Devenport M, Wang X, Zhang Y, Liu Y, Zheng P. Uncoupling therapeutic from immunotherapy-related adverse effects for safer and effective anti-CTLA-4 antibodies in CTLA4 humanized mice. Cell Res 2018; 28(4): 433–447.

109. Du X, Tang F, Liu M, Su J, Zhang Y, Wu W, Devenport M, Lazarski CA, Zhang P, Wang X, Ye P, Wang C, Hwang E, Zhu T, Xu T, Zheng P, Liu Y. A reappraisal of CTLA-4 checkpoint blockade in cancer immunotherapy. Cell Res 2018; 28(4): 416–432.

110. Liu Y, Zheng P. Preserving the CTLA-4 checkpoint for safer and more effective cancer immunotherapy. Trends Pharmacol Sci 2020; 41(1): 4–12.

111. Duell J, Lurati S, Dittrich M, Bedke T, Pule M, Einsele H, Rossig C, Topp M S. First generation chimeric antigen receptor display functional defects in key signal pathways upon antigen stimulation. Blood 2010; 116(21): 2088.

112. Carpenito C, Milone MC, Hassan R, Simonet JC, Lakhal M, Suhoski MM, Varela-Rohen A, Haines KM, Heitjan DF, Albelda SM, Carroll RG, Riley JL, Pastan I, June CH. Control of large, established tumor xenografts with genetically retargeted human T cells containing CD28 and CD137 domains. Proc Natl Acad Sci USA 2009; 106(9): 3360–3365.

113. June CH, O’Connor RS, Kawalekar OU, Ghassemi S, Milone MC. CAR T cell immunotherapy for human cancer. Science 2018; 359 (6382): 1361–1365.

114. Prasad V. Tisagenlecleucel—the first approved CAR-T cell therapy: implications for payers and policy makers. Nat Rev Clin Oncol 2018; 15(1): 11–12.

115. Bouchkouj N, Kasamon YL, de Claro RA, George B, Lin X, Lee S, Blumenthal GM, Bryan W, McKee AE, Pazdur R. FDA approval summary: axicabtagene ciloleucel for relapsed or refractory large B-cell lymphoma. Clin Cancer Res 2019; 25(6): 1702–1708.

116. Voelker R. CAR-T therapy is approved for mantle cell lymphoma. JAMA 2020; 324(9): 832.

117. Mullard A. FDA approves fourth CAR-T cell therapy. Nat Rev Drug Discov 2021; 20(3): 166.

118. Ramos CA, Grover NS, Beaven AW, Lulla PD, Wu MF, Ivanova A, Wang T, Shea TC, Rooney CM, Dittus C, Park SI, Gee AP, Eldridge PW, McKay KL, Mehta B, Cheng CJ, Buchanan FB, Grilly BJ, Morrison K, Brenner MK, Serody JS, Dotti G, Heslop HE, Savoldo B. Anti-CD30 CAR-T cell therapy in relapsed and refractory Hodgkin lymphoma. J Clin Oncol 2020; 38(32): 3794–3804.

119. Huang R, Li X, He Y, Zhu W, Gao L, Liu Y, Gao L, Wen Q, Zhong JF, Zhang C, Zhang X. Recent advances in CAR-T cell engineering. J Hematol Oncol 2020; 13(1): 86.

120. Liu Y, Guo Y, Wu Z, Feng K, Tong C, Wang Y, Dai H, Shi F, Yang Q, Han W. Anti-EGFR chimeric antigen receptor-modified T cells in metastatic pancreatic carcinoma: a phase I clinical trial. Cytotherapy 2020; 22(10): 573–580.

121. Cutmore LC, Brown NF, Raj D, Chauduri S, Wang P, Maher J, Wang Y, Lemoine NR, Marshall JF. Pancreatic Cancer UK Grand Challenge: developments and challenges for effective CAR T cell therapy for pancreatic ductal adenocarcinoma. Pancreatology 2020; 20(3): 394–408.

122. Depil S, Duchateau P, Grupp SA, Mufti G, Poirot L. ‘Off-the-shelf’ allogeneic CAR T cells: development and challenges. Nat Rev Drug Discov 2020; 19(3): 185–199.

123. Cutmore LC, Marshall JF. Current perspectives on the use of off-the-shelf CAR-T/NK cells for the treatment of cancer. Cancers (Basel) 2021; 13(18): 926.

124. Capsomidis A, Benthall G, Van Acker HH, Fisher J, Kramer AM, Abeli Z, Majani Y, Gileadi T, Wallace R, Gustafsson K, Flutter B, Anderson J. Chimeric antigen receptor-engineered human gamma delta T cells: enhanced cytotoxicity with retention of cross presentation. Mol Ther 2018; 26(3): 354–365.

125. Torikai H, Reik A, Liu PQ, Zhou Y, Zhang L, Maiti S, Huls H, Miller JC, Kebriaei P, Rabinovich B, Lee DA, Champlin RE, Bonini C, Naldini L, Rebar EJ, Gregory PD, Holmes MC, Cooper LJ. A foundation for universal T-cell based immunotherapy: T cells engineered to express a CD19-specific chimeric-antigen-receptor and eliminate expression of endogenous TCR. Blood 2012; 119 (24): 5697–5705.

126. Melenhorst JJ, Leen AM, Bollard CM, Quigley MF, Price DA, Rooney CM, Brenner MK, Barrett AJ, Heslop HE. Allogeneic virus-specific T cells with HLA alloreactivity do not produce GVHD in human subjects. Blood 2010; 116(22): 4700–4702.

127. Kochenderfer JN, Dudley ME, Carpenter RO, Kassim SH, Rose JJ, Telford WG, Hakim FT, Halverson DC, Fowler DH, Hardy NM, Mato AR, Hickstein DD, Gea-Banacloche JC, Pavletic SZ, Sportes C, Marie I, Feldman SA, Hansen BG, Wilder JS, Blacklock-Schuerer B, Jena B, Bishop MR, Gress RE, Rosenberg SA. Donor-derived CD19-targeted T cells cause regression of malignancy persisting after allogeneic hematopoietic stem cell transplantation. Blood 2013; 122(25): 4129–4139.

128. Guo F, Cui J. CAR-T in cancer treatment: develop in self-optimization, win-win in cooperation. Cancers (Basel) 2021; 13 (8): 1955.
130. Muny., Haile ST, Beinat C, Aalipour A, Alam IS, Muny., Shaffer TM, Patel CB, Graves EE, Mackall CL, Gambhir SS. Intravital imaging reveals synergistic effect of CAR T-cells and radiation therapy in a preclinical immunocompetent glioblastoma model. OncolImmunology 2020; 9(1): 1757360

131. Grozner R, Cherkasskyy L, Chintala N, Adusumilli PS. Combination immunotherapy with CAR T cells and checkpoint blockade for the treatment of solid tumors. Cancer Cell 2019; 36(5): 471–482

132. Lee YG, Marks I, Sinivasarao M, Kandhaluruk AM, Mahalingam SM, Liu X, Chu H, Low PS. Use of a single CAR T cell and several bispecific adapters facilitates eradication of multiple antigenically different solid tumors. Cancer Res 2019; 79(2): 387–396

133. Driouk L, Gicobi JK, Kamihara Y, Rutherford K, Dranoff G, Ritz J, Baumeister SHC. Chimeric antigen receptor T cells targeting NK22D-ligands show robust efficacy against acute myeloid leukemia and T-cell acute lymphoblastic leukemia. Front Immunol 2020; 11: 580328

134. Caruana I, Weber G, Ballard BC, Wood MS, Savolbo D, Dotti G. K562-derived whole-cell vaccine enhances antitumor responses of CAR redirected virus-specific cytotoxic T lymphocytes in vivo. Clin Cancer Res 2015; 21(13): 2952–2962

135. Bommarreddy PK, Shettigar M, Kaufman HL. Integrating oncolytic viruses in combination cancer immunotherapy. Nat Rev Immunol 2018; 18(8): 498–513

136. Hu Z, Ott PA, Wu CJ. Toward personalized, tumour-specific, therapeutic vaccines for cancer. Nat Rev Immunol 2018; 18(13): 168–182

137. Parkhurst MR, Yang JC, Langan RC, Dudley ME, Nathan DA, Feldman SA, Davis JL, Morgan RA, Merino MJ, Sherry RM, Hughes MS, Kammla US, Phan GQ, Lin RM, Wank SA, Restifo NP, Robbins PF, Laurencot CM, Rosenberg SA. T cells targeting carcinoembryonic antigen can mediate regression of metastatic colorectal cancer but induce severe transient colitis. Mol Ther 2011; 19(3): 620–626

138. Blass E, Ott PA. Advances in the development of personalized neoantigen-based therapeutic cancer vaccines. Nat Rev Clin Oncol 2021; 18(4): 215–229

139. Ott PA, Hu Z, Keskin DB, Shukla SA, Sun J, Bozym DJ, Zhang W, Luoma AM, Li S, Oliveira G, Giobbie-Hurder A, Felt K, Gnjini E, Shukla SA, Hu Z, Li L, Le PM, Allessec RL, Richman AR, Kowaleczky MS, Abdelrahman S, Geduldij JE, Charbonneau S, Pelton K, Iorgulescu JB, Elagia L, Zhang W, Olive O, McCluskey C, Olsen LR, Stevens I, Lane WI, Salazar AM, Daley H, Wen PY, Chiocca EA, Harden M, Lennon NJ, Gabriel S, Getz G, Lander ES, Regev A, Ritz J, Neuberg D, Rodig SJ, Ligon KL, Suvá ML, Wucherpfennig KW, Hacohen N, Fritsch EF, Livak KJ, Ott PA, Wu CJ, Reardon DA. Neoantigen vaccine generates intratumoral T cell responses in phase Ib glioblastoma trial. Nature 2019; 565 (7738): 234–239

140. Ott PA, Hu-Lieskovska S, Chmielowski B, Govindan R, Naing A, Bhaward N, Margolin K, Awad MM, Hellmann MD, Lin JJ, Friedlander T, Bushway ME, Balogk KN, Scuito TE, Kohler V, Tumblum SJ, Besada R, Curran RR, Trapp B, Scherer J, Poran A, Harjanto D, Barthelme D, Ting YS, Dong JZ, Ware Y, Huang Y, Huang Z, Wamamaker A, Cleary LD, Moles MA, Mankson K, Greshock J, Khondker ZS, Fritsch E, Rooney MS, DeMario M, Gaynor RB, Srinivasan L. A phase Ib trial of personalized neoantigen therapy plus anti-PD-1 in patients with advanced melanoma, non-small cell lung cancer, or bladder cancer. Cell 2020; 183(2): 347–362.e24

141. Keskin DB, Anandappa AJ, Sun J, Tirosh I, Mathewson ND, Li S, Oliveira G, Giobbie-Hurder A, Felt K, Gnjini E, Shukla SA, Hu Z, Li L, Le PM, Allessec RL, Richman AR, Kowaleczky MS, Abdelrahman S, Geduldij JE, Charbonneau S, Pelton K, Iorgulescu JB, Elagia L, Zhang W, Olive O, McCluskey C, Olsen LR, Stevens I, Lane WI, Salazar AM, Daley H, Wen PY, Chiocca EA, Harden M, Lennon NJ, Gabriel S, Getz G, Lander ES, Regev A, Ritz J, Neuberg D, Rodig SJ, Ligon KL, Suvá ML, Wucherpfennig KW, Hacohen N, Fritsch EF, Livak KJ, Ott PA, Wu CJ, Reardon DA. Neoantigen vaccine generates intratumoral T cell responses in phase Ib glioblastoma trial. Nature 2019; 565 (7738): 234–239

142. Ott PA, Hu-Lieskovska S, Chmielowski B, Govindan R, Naing A, Bhaward N, Margolin K, Awad MM, Hellmann MD, Lin JJ, Friedlander T, Bushway ME, Balogk KN, Scuito TE, Kohler V, Tumblum SJ, Besada R, Curran RR, Trapp B, Scherer J, Poran A, Harjanto D, Barthelme D, Ting YS, Dong JZ, Ware Y, Huang Y, Huang Z, Wamamaker A, Cleary LD, Moles MA, Mankson K, Greshock J, Khondker ZS, Fritsch E, Rooney MS, DeMario M, Gaynor RB, Srinivasan L. A phase Ib trial of personalized neoantigen therapy plus anti-PD-1 in patients with advanced melanoma, non-small cell lung cancer, or bladder cancer. Cell 2020; 183(2): 347–362.e24

143. Lindskog M, Laurrell A, Kjellman M, Melichar B, Niezabitowski J, Maroto P, Zielinski H, Villacampa F, Bigot P, Bajory ZA randomized phase II study with ilixadencel, a cell-based immune primer, plus sunitinib versus sunitinib alone in synchronous metastatic renal cell carcinoma. J Clin Oncol 2020; 38(5_suppl): 11

144. Tanyi JL, Bobisse S, Ophir E, Tuyaerts S, Roberti A, Genolet R, Baumgartner P, Stevenson BJ, Iseli C, Dangaj D, Czerniecki B, Semliotof A, Racle J, Michel A, Xenarios I, Iansan C, Chiang C, Monos D, Torigian DA, Nisenbaum HL, Michielin O, June CH, Levine BL, Powell DJ Jr, Gfeller D, Mick R, Dafni U, Zoete V, Harari A, Coukos G, Kandalaft LE. Personalized cancer vaccine effectively mobilizes antitumor T cell immunity in ovarian cancer. Sci Transl Med 2018; 10(436): eaaa5931

145. Moyañin KD, Opel CF, Szeto GL, Tzeng A, Zhu EF, Engreitz JM, Williams RT, Rakhra K, Zhang MH, Rothshields AM, Kumari S, Kelly RL, Kwan BH, Abraham W, Khu M, Mehta NK, Kauke MJ, Suh H, Cochran JR, Lauffenburger DA, Wittrup KD, Irvine DJ. Eradication of large established tumors in mice by combination immunotherapy that engages innate and adaptive immune responses. Nat Med 2016; 22(12): 1402–1410

146. Chau I, Haag G, Rahma O, Macarulla T, McCune S, Yardley D, Komm K, Drexler H, Eichler D, Deininger MW, Witkowski M, Greaves M, Jakschid M, Hogenauer C, Steigmeier W, Paule A, Canellos G, Kandalaft LE. Personalized cancer vaccine effectively mobilizes antitumor T cell immunity in ovarian cancer. Sci Transl Med 2018; 10(436): eaaa5931

147. Simonsen KL, Fracasso PM, Bernstein SH, Wind-Rotolo M, Gupta M, Comprelli A, Reilly TP, Cassidy J. The Fast Real-time Assessment of Combination Therapies in Immuno-ONcology (FRACT) program: innovative, high-throughput clinical screening of immunotherapies. Eur J Cancer 2018; 103: 259–266

148. Redman JM, Steinberg SM, Gulley JL. Quick efficacy seeking trial (QuEST1): a novel combination immunotherapy study designed...
for rapid clinical signal assessment metastatic castration-resistant prostate cancer. J Immunother Cancer 2018; 6(1): 91

149. Tang J, Shalabi A, Hubbard-Lucey VM. Comprehensive analysis of the clinical immuno-oncology landscape. Ann Oncol 2018; 29(1): 84–91

150. Monk BJ, Brady MF, Aghajanian C, Lankes HA, Rizack T, Leach J, Fowler JM, Higgins R, Hanjani P, Morgan M, Edwards R, Bradley W, Kolevska T, Foukas P, Swisher EM, Anderson KS, Gottardo R, Bryan JK, Newkirk M, Manjarrez KL, Mannel RS, Hershberg RM, Coukos G. A phase 2, randomized, double-blind, placebo-controlled study of chemo-immunotherapy combination using motolimod with pegylated liposomal doxorubicin in recurrent or persistent ovarian cancer: a Gynecologic Oncology Group partners study. Ann Oncol 2017; 28(5): 996–1004

151. Yu S, Yi M, Qin S, Wu K. Next generation chimeric antigen receptor T cells: safety strategies to overcome toxicity. Mol Cancer 2019; 18(1): 125

152. Levy BP, Giaccone G, Besse B, Felip E, Garassino MC, Domine Gomez M, Garrido P, Piperdi B, Ponce-Aix S, Menezes D, MacBeth KJ, Risueño A, Slepetis R, Wu X, Fandi A, Paz-Ares L. Randomised phase 2 study of pembrolizumab plus CC-486 versus pembrolizumab plus placebo in patients with previously treated advanced non-small cell lung cancer. Eur J Cancer 2019; 108: 120–128

153. Mijalis AJ, Thomas DA 3rd, Simon MD, Adamo A, Beaumont R, JensenKF, Pentelute BL. A fully automated flow-based approach for accelerated peptide synthesis. Nat Chem Biol 2017; 13(5): 464–466