CD3-Bispecific Antibody Therapy

Subjects: Oncology

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CD3-bispecific antibody therapy is a form of immunotherapy that enables soldier cells of the immune system to recognize and kill tumor cells.

antibody therapy | immuno-oncology | CD3-bispecific antibody

1. Introduction

CD3-bispecific antibodies (CD3-BsAbs) are an emerging treatment modality in the field of cancer immunotherapy. BsAbs can recognize distinct antigens with each of their antigen-binding domains, in contrast to conventional Abs that recognize the same antigen with both Fab arms. The exception is IgG4, which has been reported to naturally exchange arms to attain bispecificity [1]. CD3-BsAbs act by simultaneous binding to a tumor-associated antigen (TAA) expressed on tumor cells and to CD3 on a T cell (CD3xTAA) [2]. Crosslinking of these two cell types by CD3-BsAbs allows the formation of an immunological synapse, similar to that of a natural T-cell receptor (TCR)/peptide–major histocompatibility complex (MHC) complex [3]. This synapse results in T-cell activation and thereby the secretion of inflammatory cytokines and cytolytic molecules that are able to kill the tumor cells in the process. The strength of CD3-BsAbs lies in the fact that any T cell could serve as an effector cell, regardless of TCR specificity, as for these BsAbs, TCR signaling does not require engagement of the antigen-binding domain of the TCR, but is initiated via CD3 [4]. Therefore, CD3-BsAbs can employ all available T cells and are not limited to tumor-specific T cells, contrary to the key requirement for effective immune checkpoint therapy [5].

CD3-BsAb therapy is a passive form of immunotherapy and shows striking kinship with the adoptive cell transfer of T cells expressing chimeric antigen receptor (CAR) transgenes [6]. CARs consist of TAA binding domains from antibodies directly linked to the intracellular CD3ζ chain and domains from costimulatory receptors (e.g., 4-1BB) and thereby activate T cells upon antigen recognition. CD3-BsAbs and CAR T cells are similar in many ways: both target a surface TAA, both exploit T-cell effector functions and both are successfully used in the clinic for hematological malignancies and show a similar type of toxicity profile [7][8]. Some disadvantages of currently clinically approved CAR T cells compared to CD3-BsAbs are: (1) patients are required to be lymphodepleted prior to infusion of CAR T cells, (2) CAR T cells have to be individually produced for each patient, whereas CD3-BsAbs can serve as off-the-shelf therapeutics, (3) CAR T cells remain in the patients after the tumor is cleared, resulting in continuous B-cell depletion in the case of CD19-targeting CAR T cells, whereas CD3-BsAbs are cleared from the blood over time and (4) unlike CD3-BsAbs, dosing cannot be adjusted to minimize adverse events [7][9]. Nevertheless, it will be important to learn from the CAR T cell field to potentially extrapolate new findings to the CD3-BsAb field.

Over the last few years, new insights in BsAb biology and enabling technologies resulted in the generation of many different formats of CD3-BsAbs, which was elaborately reviewed by Labrijn et al. [10]. As of December 2020, over 100 different CD3-BsAb formats are known, ranging from very small fragments containing two different variable domains without an Fc tail, conventional antibody structures (two Fab arms linked to an Fc tail) and larger structures with additional variable domains linked to the conventional antibody structure. These different formats determine important features, such as antibody half-life via neonatal Fc receptor (FcRn)-mediated recycling, immunogenicity, type of effector response via altered immune synapse formation and ability to penetrate in solid tumors [11]. The presence and functionality of the Fc tail determines whether the BsAb is able to bind to and activate Fc receptor (FcR)-expressing immune cells, which could lead to stronger inflammatory responses, but also allows activation of immune cells in the absence of TAA, potentially resulting in more severe adverse events (AEs) [12].

Currently, CD3-BsAbs show great potential for hematological cancers, with the FDA-approved blinatumomab (CD3xCD19) being successfully used in the clinic to treat some B-cell malignancies. Many other CD3-BsAbs are being tested in (pre)clinical studies for both hematological and solid tumors. However, contrary to the success of CD3-BsAbs in hematological malignancies, the effect of these antibodies in solid tumors is still rather limited [13].

2. CD3-BsAbs in Hematological Malignancies

CD3-BsAbs received a lot of attention due to their success in hematological cancers. Blinatumomab (a CD3xCD19 BsAb without an Fc tail) was FDA approved in 2014 and is now successfully used in the clinic to treat patients suffering from relapsed or refractory B-cell precursor acute lymphoblastic leukemia (ALL) [14]. Over 40% of adult patients treated with blinatumomab show a complete or partial response and median overall survival is improved by several months compared to standard of care chemotherapy [15][16][17]. Unfortunately, most patients still relapse eventually after primary response to blinatumomab therapy. These relapses are currently being extensively investigated and the data have thus far indicated that relapses are frequently found at immune-privileged extramedullary locations and some relapses have lost CD19 antigen expression, but more research is required to further elucidate these resistance mechanisms [18][19].

Apart from blinatumomab, many other CD3-BsAbs are currently in clinical trials targeting well-established B-cell markers, like CD19, CD20, CD38 and B-cell maturation antigen (BCMA) and myeloid markers, like CD33 and CD123. For instance, in a phase I/II study, patients suffering from acute myeloid leukemia (AML) were treated with flotetuzumab (CD3xCD123 BsAb) and showed promising overall response rates (complete response with full, partial or incomplete recovery of blood cells) of 30% [20]. In another phase I/II study for patients suffering from diffuse large B-cell lymphoma (DLBCL), high-grade B-cell lymphoma (HGBCL) or follicular lymphoma (FL), epcoritamab (CD3xCD20 BsAb) therapy generated impressive responses: 44% complete response (CR) and 11% partial response (PR) for patients with DLBCL or HGBCL and 100% PR for patients with FL [21]. Comparable results were obtained with other CD3xCD20 bispecifics [22][23]. In NOD/SCID-gamma null (NSG) mice, REGN1979 (CD3xCD20) delayed tumor outgrowth better than rituximab, thereby further indicating the strength of CD3-BsAbs [24]. Interestingly, some of these trials target the same B-cell or myeloid antigens, however, with different CD3-BsAb

formats. Therefore, these clinical studies could potentially inform on the role of different antibody formats' treatment safety and efficacy.

Clinical trials with blinatumomab revealed that cytokine release syndrome (CRS) is one of the major safety-related AEs [25]. The availability of $CD19^+$ tumor cells and healthy B- and T cells in the same compartment allows acute and synchronic CD3-BsAb-mediated T-cell activation, followed by excessive release of inflammatory cytokines, such as IFN-γ, IL-6 and TNF-α, resulting in symptoms ranging from mild fever to multi-organ system failure [26]. However, CRS is not a specific problem for blinatumomab, but is observed for all CD3-BsAbs and CAR T-cell therapies in both hematological and solid cancer indications with CRS severities dependent on the type of therapy and target [27][28]. Preclinical research using a humanized mouse model showed that the primary mediator of CD3-BsAb-induced CRS was TNF-α produced by activated T cells, leading to massive secretion of inflammatory cytokines by monocytes [29]. The blockade of upstream TNF-α and downstream IL-1β or IL-6 can mitigate CRS [29][30][31]. Others have reported that step-up dosing, or subcutaneous administration of CD3-BsAbs, decreased the extent of CRS [32][33]. Furthermore, several preclinical studies in mouse and cynomolgus monkey models showed that reducing CD3 affinity could reduce treatment-induced cytokine levels [34][35][36][37].

3. Historical Perspective and Current Status of CD3-BsAbs in Solid Cancers

Despite the fact that CD3-BsAbs are mostly known for their use in hematological malignancies, the first European medicines agency (EMA)-approved CD3 bispecific antibody was catumaxomab, a CD3xEpCAM BsAb for the intraperitoneal treatment of epithelial cell adhesion molecule (EpCAM)-positive malignant ascites [38]. This antibody was actually trifunctional, as its Fc was able to bind FcR-expressing cells and induced strong immunological responses [39]. Severe liver toxicity was also observed due to the activation of Kupffer cells when administered intravenously [12]. Catumaxomab was eventually withdrawn for commercial reasons in 2017, but taught the field an important lesson about the potential dangers of the presence of an active Fc in CD3-BsAbs. All current full length CD3-BsAbs in development contain Fc-silent backbones with mutations impairing the binding of FcγR and C1q [10]. Moreover, preclinical studies showed that Fc-silenced full length CD3-BsAbs improved T-cell trafficking towards the tumor and induced better anti-tumor responses. Wang et al. showed that CD3-BsAbs with an active Fc backbone failed to drive T cells to the tumor, but instead induced either T-cell depletion or the accumulation of T cells in the lungs [40]. This observed effect was attributed to the capacity of the Fc backbone to be bound by FcγR-expressing myeloid cells. Fc-silenced CD3-BsAbs did not lead to sequestration of T cells in the lungs, but they arrived in the tumor. More importantly, therapeutic efficacy was greatly improved in Fc-silenced CD3-BsAb-treated mice. A similar trend was also observed in a syngeneic mouse model, where CD3xTrp1 (tyrosinase-related protein 1) was used to treat Trp1-positive B16F10 tumor cells [41].

As of December 2020, no CD3-BsAbs are approved for the treatment of solid tumors in the clinic. However, many different targets are being explored in clinical studies, of which most are focusing on classical TAAs, such as carcinoembryonic antigen (CEA), epidermal growth factor receptor (EGFR), EpCAM, HER2 and prostate-specific membrane antigen (PSMA). Other TAAs are also being explored (see Table 1 for an elaborate list). Most of these

studies simply inject CD3-BsAbs, however, in some studies, these Abs piggyback with infused T cells as “bispecific-armed T cells”. Furthermore, this table also includes CD3-BsAb formats based on affinity-enhanced TCR-like domains that recognize peptide–human leukocyte antigen (HLA) complexes (immune mobilizing monoclonal T-cell receptors against cancer (ImmTACs)) [42]. Multiple other TAAs are currently pursued in preclinical studies hoping to make their way to the clinic, including B7-H4, CD133, CD155, claudin 6 (CLDN6), cellular mesenchymal to epithelial transcription factor (C-MET), ephrin receptor A10 (EphA10), folate receptor 1 (FOLR1), HLA-A*24:survivin $2B_{80-88}$, integrin β4 (ITGB4), P-cadherin, prolactin receptor (PRLR), receptor tyrosine kinase-like orphan receptor 1 (ROR1), TNF-related apoptosis-inducing ligand receptor (TRAIL-R2), transferrin receptor (TfR) and tumor-associated calcium signal transducer 2 (Trop-2) [43][44][45][46][47][48][49][50][51][52][53][54][55][56][57][58][59][60][61][62][63][64][65][66][67][68][69].

Table 1. Overview of clinical studies involving CD3-BsAbs targeting solid tumors.

TAA	Disease	Phase
Completed Clinical Trials		
CEA	CEA-positive tumors	Phase I (NCT02324257, completed)
CEA	Gastrointestinal adenocarcinomas	Phase I NCT01284231, completed)
CEA	Advanced CEA-positive solid tumors	Phase I (NCT02291614, completed)
CEA	Advanced CEA-positive solid tumors	Phase I (NCT02650713, completed)
EGFR	Brain and central nervous system tumors	Phase I (NCT00005813, completed)
EpCAM	Solid tumors	Phase I (NCT00635596, completed)
EpCAM	Ascites, ovarian cancer, fallopian tube cancer, peritoneal cancer	Phase II (NCT00326885, completed)
EpCAM	Ovarian cancer, fallopian tube cancer, peritoneal cancer	Phase II (NCT00377429, completed)
EpCAM	Recurrent ovarian cancer, fallopian tube cancer, peritoneal carcinomatosis	Phase II (NCT01815528, completed)
EpCAM	Ovarian cancer, fallopian tube cancer, peritoneal cancer	Phase II (NCT01246440, completed)
EpCAM	Ovarian cancer	Phase II (NCT00563836,

TAA	Disease	Phase
		completed)
EpCAM	Ascites, carcinoma, epithelial cancer	Phase II (NCT01065246, completed)
EpCAM	Ovarian cancer, gastric cancer, pancreatic cancer, malignant ascites	Phase II (2005-001700-39, completed)
EpCAM	Gastric cancer and gastric adenocarcinoma	Phase II (NCT00352833, completed)
EpCAM	Peritoneal carcinomatosis and gastric adenocarcinoma	Phase II (NCT01504256, completed)
EpCAM	Ovarian cancer, fallopian tube cancer, peritoneal cancer	Phase II (NCT00189345, completed)
EpCAM	Gastric cancer and gastric adenocarcinoma	Phase II (NCT00464893, completed)
EpCAM	Malignant ascites and EpCAM-positive tumors	Phase II/III, (NCT00836654, completed)
EpCAM	EpCAM-positive solid cancers	Phase III (NCT00822809, completed)
GD2	Neuroblastoma	Phase I (NCT00877110, completed)
gpA33	Colorectal carcinoma	Phase I (NCT02248805, completed)
GPC3	Solid tumors	Phase I (NCT02748837, completed)
HER2	Breast cancer, metastatic breast cancer	Phase I (NCT00027807, completed)
HLA-A*02:01:gp100	Melanoma, advanced melanoma	Phase I (NCT01209676, completed)
HLA-A*02:01:gp100	Malignant melanoma	Phase I (NCT01211262, completed)
PSMA	Prostate cancer	Phase I (NCT02262910, completed)
PSMA	Prostatic neoplasms	Phase I (NCT01723475, completed)
Active clinical trials *		

TAA	Disease	Phase
5T4	Malignant solid tumors	Phase I/II (NCT04424641, recruiting)
B7-H3	Advanced solid tumors, metastatic solid tumors	Phase I (NCT03406949, active not recruiting)
CEA	Colorectal cancers	Phase I (NCT03866239, recruiting)
CEA	NSCLC	Phase I/II (NCT03337698, recruiting)
CEA, EGFR, GPC3, HER2, MUC1	Malignant solid tumors	Phase I (NCT04076137, recruiting)
CEA, EpCAM, GPC3, MUC1	Advanced liver cancer	Phase II (NCT03146637, recruiting)
CLDN18.2	Gastric and gastroesophageal junction adenocarcinoma	Phase I (NCT04260191, recruiting)
DLL3	Small cell lung carcinoma	Phase I (NCT03319940, recruiting)
DLL3	Small cell lung cancer, advanced cancers	Phase I/II (NCT04471727, not yet recruiting)
EGFR	Multiple solid gastrointestinal tumors	Phase I (NCT01420874, active not recruiting)
EGFR	Glioblastoma multiforme, gliosarcoma	Phase I (NCT03344250, recruiting)
EGFR	Pancreatic cancer	Phase I (NCT04137536, recruiting)
EGFR	Advanced pancreatic cancer	Phase Ib/II (NCT02620865, active not recruiting)
EGFR	Advanced and metastatic pancreatic adenocarcinoma	Phase Ib/II (NCT03269526, recruiting)
EGFRv3	Glioblastoma multiforme, malignant glioma	Phase I (NCT03296696, active not recruiting)
EpCAM	Large bowel (colon) cancer, colorectal cancer	Phase U (ChiCTR-ROC-16008620, not yet recruiting)
EpCAM	Malignant ascites, advanced solid tumors	Phase I (CTR20181212, recruiting)

TAA	Disease	Phase
EpCAM	Ascites, advanced solid tumors	Phase I (ChiCTR1900024144, recruiting)
EpCAM	Malignant ascites	Phase I (NCT04501744, recruiting)
EpCAM	Gastric adenocarcinoma, peritoneal carcinomatosis, colorectal adenocarcinoma	Phase II (2010-022810-26, recruiting)
EpCAM	Advanced gastric cancer, stomach cancer, gastric cancer	Phase III (NCT04222114, recruiting)
GD2	Neuroblastoma	Phase I (NCT02650648, active not recruiting)
GD2	Neuroblastoma, osteosarcoma, other solid tumors	Phase I/II (NCT03860207, recruiting)
GD2	Neuroblastoma, osteosarcoma	Phase I/II (NCT02173093, recruiting)
gpA33	Metastatic colorectal cancer	Phase I/II (NCT03531632, active not recruiting)
GPC3	Advanced solid tumors, recurrent solid tumors	Phase I (JapicCTI-194805, recruiting)
GUCY2C	Gastrointestinal malignancies, esophageal cancer	Phase I (NCT04171141, recruiting)
HER2	Breast cancer	Phase U (ChiCTR-ROC-16008650, not yet recruiting)
HER2	HER2-positive solid tumors	Phase I (NCT04501770, recruiting)
HER2	Breast cancer and leptomeningeal metastases	Phase I (NCT03661424, recruiting)
HER2	Esophageal, gastric, pancreatic, liver, gallbladder and bowel cancer	Phase I (NCT02662348, unknown status)
HER2	Advanced solid tumors	Phase I (NCT03448042, recruiting)
HER2	Advanced solid tumors	Phase I (CTR20171194, recruiting)
HER2	Solid tumors, advanced solid tumors	Phase I (ChiCTR1900024128, recruiting)

TAA	Disease	Phase
HER2	Breast cancer	Phase I/II (NCT03983395, recruiting)
HER2	Metastatic breast cancer	Phase I/II NCT03272334, recruiting)
HER2	Metastatic castration resistant prostate cancer	Phase II (NCT03406858, status unknown)
HER2	Breast cancer	Phase II (NCT01147016, status unknown)
HER2	Breast cancer	Phase II (NCT01022138, status unknown)
HLA-A*02:01:gp100	Uveal melanoma	Phase I/II (NCT02570308, active not recruiting)
HLA-A*02:01:gp100	Melanoma	Phase I/II (NCT02535078, active not recruiting)
HLA-A*02:01:gp100	Uveal melanoma, metastatic uveal melanoma, advanced uveal melanoma	Phase II (NCT03070392, active not recruiting)
HLA-A*02:MAGE-A4	Advanced solid tumors, metastatic solid tumors	Phase I/II (NCT03973333, recruiting)
MSLN	Mesotheliomas, ovarian cancers, pancreatic cancers	Phase I/II (NCT03872206, recruiting)
MUC16	Ovarian cancer fallopian tube cancer, peritoneal cancer	Phase I/II (NCT04590326, not yet recruiting)
MUC16	Ovarian cancer fallopian tube cancer, peritoneal cancer	Phase I/II, (NCT03564340, recruiting)
MUC17	Gastric and gastroesophageal junction cancer	Phase I (NCT04117958, recruiting)
NY-ESO1	NY-ESO1-positive tumors	Phase I/II (NCT03515551, recruiting)
PRAME	Advanced solid tumors, cancer indications	Phase I/II (NCT04262466, recruiting)
PSCA	NSCLC, breast cancer, pancreatic cancer, urogenital cancer	Phase I NCT(03927573, recruiting)
PSMA	Prostate cancer	Phase I (NCT04077021, recruiting)

* Data as of 13 November 2020. Clinical studies are ordered based on the targeted tumor-associated antigen (TAA). CEA, carcinoembryonic antigen; CLDN18.2, claudin18 isoform 2; DLL3, delta-like ligand 3; EGFR,

TAA	Disease	Phase
PSMA	Prostate cancers, advanced solid tumors, neoplasms, renal cancers, small cell lung cancer	Phase I (NCT03926013, recruiting)
PSMA	Castration-resistant prostate carcinoma	Phase I (NCT04104607, recruiting)
PSMA	Metastatic castration-resistant prostate cancer	Phase I (NCT03792841, recruiting)
PSMA	Squamous cell lung carcinoma	Phase I/II NCT04496674, not yet recruiting)
PSMA	Prostate cancer	Phase I/II (NCT03577028, recruiting)
SSTR2	Neuroendocrine tumors and gastrointestinal neoplasms	Phase I (NCT03411915, recruiting)
SSTR2	Merkel cell carcinoma and small cell lung cancer	Phase I/II (NCT04590781, not yet recruiting)
STEAP1	Metastatic castration-resistant prostate cancer	Phase I (NCT04221542, recruiting)

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responders, of which one had marked regression of soft tissue and bone metastases. Overall, some evidence for efficacy induced by CD3-BsAbs in solid tumors has been found, however, with only a handful of long-term survivors, some partial responses and the occurrence of multiple DLTs, the development of CD3-BsAbs in solid tumors lags behind that in hematological malignancies.

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