Molecular Oncology

FEBS PRESS science publishing by scientists

COMMENTARY

Bispecific antibodies come to the aid of cancer immunotherapy

Ivano Amelio[1,2], Gerry Melino[1] and Arnold J. Levine[3]

1 Department of Experimental Medicine, University of Rome Tor Vergata, TOR, Rome, Italy
2 School of Life Sciences, University of Nottingham, Nottingham, UK
3 Institute for Advanced Study, Simons Center for Systems Biology, Princeton, NJ, USA

Correspondence
I. Amelio, G. Melino, Department of Experimental Medicine, University of Rome Tor Vergata, TOR, Rome, Italy
E-mail: ivano.amelio@uniroma2.it (IA); melino@uniroma2.it (GM)
A. J. Levine, Institute for Advanced Study, Simons Center for Systems Biology, Princeton, NJ, USA
E-mail: alevine@ias.edu

(Received 6 April 2021, revised 25 April 2021, accepted 30 April 2021, available online 14 May 2021)

doi:10.1002/1878-0261.12977

The use of immune checkpoint inhibitors (ICI) to activate adaptive immune responses against some tumor types has clearly been a major advance in cancer therapeutics [1]. Despite the significant impact of ICI implementation on the outcomes of cancers, treatments with ICI currently fail to eradicate the majority of tumors as a result of inadequate priming of an autologous tumor-specific immune response. Three collaborative studies published by the groups of B. Vogelstein, S. B. Gabelli, and S. Zhou indicate that specially designed bispecific antibodies may help overcome limitations of ICI.

For an adaptive immune response to develop, intracellular proteins are processed by proteolysis into peptides. Antigenic peptides are then loaded onto transporters that move the peptide antigen to a receptor, which will present the antigen on the cell surface. These receptors are genetically encoded by a chromosome 6 region of highly repetitive DNA sequences, termed the major histocompatibility complex locus (MHC). In the human population, the MHC locus contains a large number of polymorphic genes encoding human leukocyte antigen (HLA) class I and class II molecules. Mutated peptides (neo-peptides)—commonly derived from genetic missense mutations that accumulate during the process of tumor transformation—are loaded onto HLA class I and class II receptors and presented on the surface of cancer cells [2]. Tumor antigens are commonly classified as tumor-associated antigens (TAAs, such as alpha-fetoprotein) and tumor-specific antigens (TSAs, such as neoantigens). The former are aberrantly expressed at high levels by cancer cells, but are also expressed by a subset of normal cells; hence, they are not absolutely specific to the cancer tissues. Conversely, TSAs are specifically found only in cancer cells, as they generally derive from gene products with somatic mutations in the coding region of the gene.

Priming autologous CD4 and CD8 T-cell responses against tumors may fail due to several reasons: loss of cell surface HLA receptors; lack of TSAs, especially in tumors with a low tumor mutational burden; establishment of an immunosuppressive tumor microenvironment through the presence of myeloid-derived suppressor cells; CD8 T-cell exhaustion; and impaired co-localization and interaction of CD4 T cells, CD8 T cells, and antigen-presenting cells [2–5].

Aiming at boosting tumor-specific immune responses by improving immune cell co-localization and interaction, B. Vogelstein, S. B. Gabelli, S. Zhou, and colleagues have developed bispecific antibodies that couple cells harboring HLA-presented TSAs from a Tp53 mutant allele (R175H; Hsiue *et al.* [6]) or a Ras mutant (G12V, and Q61H, Q61R, or Q61L; Douglass *et al.* [7]) with CD4 and CD8 T cells. This work has been expanded in a third research project by the same teams, where the authors employed a similar concept to treat T-cell malignancies with bispecific antibodies

Abbreviations
HLA, human leukocyte antigen; ICI, immune checkpoint inhibitors; MHC, major histocompatibility complex locus; scDb, single-chain diabody; scFv, single-chain variable fragment; TAAs, tumor-associated antigens; TSAs, tumor-specific antigens.

designed to bring together malignant T cells expressing a particular clonal TCR beta-antigen with nonmalignant helper CD4 and killer CD8 T cells (Paul *et al.* [8]). These studies demonstrate that improved immune cell co-localization and their interaction with tumor antigen-presenting cells are required for the selective immune-mediated killing of tumors.

The specially designed bispecific antibodies constituted from one antibody receptor that binds with high affinity to the mutant peptide–HLA complex on cancer cells, but not to its wild-type counterpart on normal cells, and one antibody receptor that binds with the T-cell receptor–CD3 complex on T cells. This antibody construct could successfully couple, in the same locality of the tumor or draining lymph nodes, the TSA presentation complex with T cells, leading to T-cell activation and clonal expansion. Subsequently, TSA-specific T cells were shown to secrete cytokines that indirectly aid cancer cell killing, while CD8 T cells directly attacked the tumor (Fig. 1). The Tp53 and Ras mutations targeted by these bispecific antibodies are among the most common missense mutations specific to cancer cells and, thereby, constitute excellent targets for priming tumor-specific immune responses. However, only selected polymorphic HLA class I and class II receptors bind to Tp53 R175H or K-Ras G12V mutant peptides [9] and they do so with a poor affinity, limiting the applicability of treatment with p53 R175H or K-Ras G12V-targeting bispecific antibodies to a subset of patients. Nevertheless, the authors have focused their bispecific antibodies upon the HLA-A1 or HLA-A3 series of receptors and have engineered antibody affinities so that even low levels of antigens can be detected.

To target the neoantigen derived from the *TP53* mutation R175H (arginine-to-histidine 175 substitution)[10,11], Hsiue *et al.* conducted a phage display positive selection of naïve human antibody libraries against HLA-A*02:01 peptide–HLA monomers that contained the p53 R175H peptide, combined with a negative selection against peptide–HLA monomers containing the p53 wild-type peptide. The selected phage clones were then converted into a bispecific antibody, linking in a single-chain diabody (scDb) format each individual single-chain variable fragment (scFv) to an anti-CD3e scFv (UCHT1), which confers the ability to bind CD3 and activates a polyclonal T-cell response. Specificity of the top ranked phage peptide binder scDbs (including H2-scDb) for p53 R175H was further proven using functional assays assessing specific binding to cells expressing p53R175H. Importantly, scDb did neither bind to cells expressing wild-type p53 proteins, nor bind to cell lines expressing other p53 missense mutations and cell lines where p53 R175H expression was deleted using CRISPR-mediated genome editing. These results indicated low risk of cross-reactivity and off-target effects. X-ray crystallography studies shed light onto the structural basis of scDb binding to mutated p53R175H/HLA-A*02:01, further supporting target selectivity. H2-scDb selectively killed p53 R175H cancer cells *in vitro* and *in vivo* in a polyfunctional T-cell-dependent manner. No treatment effect was observed following administration of H2-scDb in NOD-SCID-Il2rg$^{-/-}$ mice engrafted with p53 R175H, but in the absence of combination engraftment with human T cells [6].

An analogous strategy and experimental validation process were employed to develop scDbs targeting HLA alleles conjugated to RAS G12V and RAS Q61H, RAS Q61L, or RAS Q61R. Douglass *et al.* [7] identified scDbs displaying strong selectivity and high affinity against cancer cells expressing low levels of specific RAS mutants. Cross-reactivity to other human peptides of similar or related composition cannot yet be excluded. After all both p53 and *Ras* belong to gene families (*p53, p63, and p73* [12–16] and *H-ras, K-ras, and N-ras* [17]) that are expressed widely in diverse cell types. Hopefully, the high affinity reported for the bispecific antibody will also translate into high specificity when the bispecific antibodies are administered to patients.

A slightly different strategy was followed to develop scDbs against T-cell malignancies. Targeting T-cell cancers with antibodies that kill all healthy and malignant T cells is not a viable therapeutic option as healthy T cells are required for a functioning immune system. Hence, therapeutic antibodies need to eradicate malignant T cells while sparing the majority of nonmalignant T cells. The TCR β-chain variable regions can be composed through the rearrangement of 30 possible *TRBV* family members, and in clonal T-cell malignancies, only a single TRBV region is expressed on the surface of the malignant T cells, while polyclonal nonmalignant T cells express a range of different TCR β-family members. Thus, TCR b-chain expressed by malignant T cells represents an ideal TAA for therapeutic applications. As proof-of-concept, Paul *et al.* developed scDbs against TRBV5-5 or TRBV12, tethered to a CD3-specific antibody. These agents could selectively eliminate malignant human T cells transplanted into mice, preserving the majority of healthy human T cells, which did not express the targeted TRBV. The authors did not select the targeting antibody with a phage display experimental procedure but employed previously identified TRBV5-5 and TRBV12-specific antibodies [8]. Of note,

Molecular Oncology **15** (2021) 1759–1763 © 2021 The Authors. Molecular Oncology published by John Wiley & Sons Ltd on behalf of Federation of European Biochemical Societies.

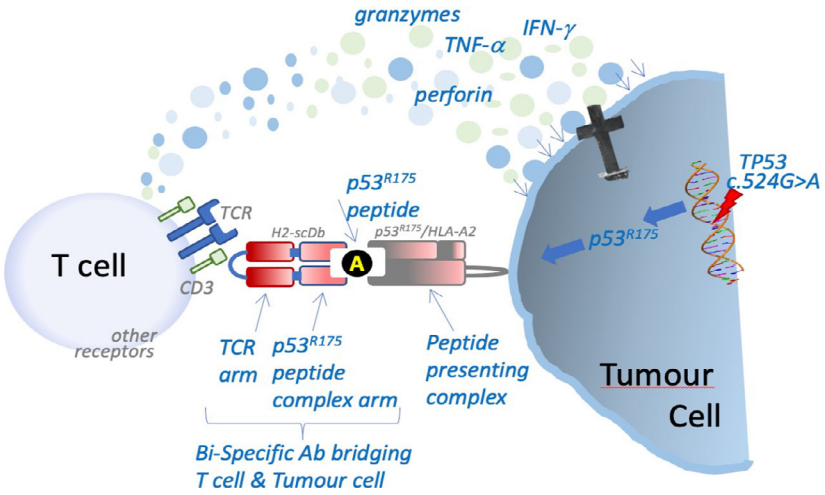

Fig. 1. Schematic representation of the mechanism of action of single-chain diabodies against p53 R175H. Antigenic peptides including the R175H mutant epitope of p53 are exposed on the surface of the cancer cells bound to the HLA complex. A scDb such as H2-scDB, binding *via* its one arm to the antigen-presenting HLA complex on the cancer cell and *via* its other arm to the CD3/TCR complex on T cells, thereby bringing T cells in close proximity with tumor antigen-specific tumor cells. Subsequent T-cell activation results in killing of the cancer cell, mediated through the release of cytotoxic molecules. CD3, cluster of differentiation 3; TCR, T-cell receptor; TNF-α, tumor necrosis factor-alpha; IFN-γ, interferon-gamma.

bispecific antibodies have previously been used to develop and target aberrantly expressed, nonmutated, cancer proteins (TAAs)[18].

Collectively, these publications demonstrate that targeting TSAs (mutated oncogenes/oncosuppressors) with bispecific antibodies is a reasonable approach to cancer therapy in humans. TSAs presented in the context of a specific HLA type have been targeted previously by *in vitro* T-cell expansion and transfer back into patients [19]. A disadvantage of that approach, compared with the use of scDbs, is that adaptive cell therapies require a time-consuming and expensive set of manipulations of patient-derived immune cells. The use of scDbs could conversely represent an 'off-the-shelf' pre-prepared easily accessible and, thus, widely applicable therapeutic set of reagents.

The significance of the studies by B. Vogelstein, S. B. Gabelli, S. Zhou, and colleagues lies within the formal and high-quality demonstrations of the feasibility of bispecific antibody design that ensures the high affinity that is required for targeting and eliminating cancer cells with good specificity (recognizing between mutant *vs* wild-type TSAs in the HLA pocket). The ability to achieve such a high affinity and specificity could switch the emphasis of cancer immunotherapy approaches from some cell-based strategies to clinical implementation of bispecific antibodies. Nevertheless, the route to taking these bispecific antibodies into the clinic is long, as several issues remain to be evaluated. Previous trials with some bispecific antibodies have triggered an overexpression of innate immune system cytokines (such as IL-6) resulting in toxic shock or organ failures [2]. Other responses to bispecific antibodies that liberate transforming growth factor-beta and IL-10 can give rise to CD4 regulatory (Treg) cells that suppress the immune system instead of activating it [2]. The immune system is well known to change with the age of the patient, or past environmental history, and is sexually dimorphic. One may well expect to obtain different responses to the manipulation of the immune system based upon age, sex, or past exposures to the microbiome or chemical or physical interventions [20–24]. Many tumors deploy an immunosuppressive repertoire of signaling molecules, and how scDbs will function in this landscape is an open question. Indeed, mutations in the p53 pathway themselves, activated by *Ras* mutations, alter the innate immune responses of some cells (senescence-activated secretory proteins) changing the expected immune activities [2]. The pairing of K-ras and Tp53 mutations in some cancers is quite common because a *Ras* mutation activates p53 activity. It would be of some interest for the authors to explore the treatment of these cancers using both bispecific antibodies to target K-ras and Tp53 mutations simultaneously.

In spite of these difficulties, we need to learn how to arm the immune system so that it truly eliminates tumors or, even better, prevents tumors from arising.

Until then, it seems unlikely that newly developed small molecules or drugs will efficiently eliminate tumors in the absence of a tumor-specific immune response [25]. So, in spite of the long list of difficulties in testing immune-altering reagents in humans we must go on to obtain a deeper understanding of the complexities of the homeostatic immune system of humans.

Acknowledgements

This work has been supported by the Associazione Italiana per la Ricerca contro il Cancro (AIRC) to IA (AIRC Start-Up ID 23219; 2020-2024) and to GM (IG#20473; 2018-2022).

Conflict of interest

The authors declare no conflict of interest.

References

1 Topalian SL, Taube JM, Anders RA & Pardoll DM (2016) Mechanism-driven biomarkers to guide immune checkpoint blockade in cancer therapy. *Nat Rev Cancer* **16**, 275–287.
2 Gupta RG, Li F, Roszik J, & Lizée G (2021) Exploiting tumor neoantigens to target cancer evolution: current challenges and promising therapeutic approaches. *Cancer Discov* **11**, 1024–1039.
3 Messmer MN, Snyder AG & Oberst A (2019) Comparing the effects of different cell death programs in tumor progression and immunotherapy. *Cell Death Differ* **26**, 115–129.
4 Amelio I, Bertolo R, Bove P, Candi E, Chiocchi M, Cipriani C, Di Daniele N, Ganini C, Juhl H, Mauriello A *et al* (2020) Cancer predictive studies. *Biol Direct* **15**. http://dx.doi.org/10.1186/s13062-020-00274-3.
5 Larmuseau M, Verbeke LPC & Marchal K (2019) Associating expression and genomic data using co-occurrence measures. *Biol Direct* **14**, 10.
6 Hsiue EH, Wright KM, Douglass J, Hwang MS, Mog BJ, Pearlman AH, Paul S, DiNapoli SR, Konig MF, Wang Qing *et al* (2021) Targeting a neoantigen derived from a common TP53 mutation. *Science* **371**. http://dx.doi.org/10.1126/science.abc8697
7 Douglass J, Hsiue EH, Mog BJ, Hwang MS, DiNapoli SR, Pearlman AH, Miller MS, Wright KM, Azurmendi PA, Wang Q *et al* (2021) Bispecific antibodies targeting mutantRASneoantigens. *Sci Immunol* **6**. http://dx.doi.org/10.1126/sciimmunol.abd5515
8 Paul S, Pearlman AH, Douglass J, Mog BJ, Hsiue EH, Hwang MS, DiNapoli SR, Konig MF, Brown PA, Wright KM *et al* (2021) TCR β chain–directed bispecific antibodies for the treatment of T cell cancers. *Sci Transl Med* **13**. http://dx.doi.org/10.1126/scitranslmed.abd3595
9 Malekzadeh P, Pasetto A, Robbins PF, Parkhurst MR, Paria BC, Jia L, Gartner JJ, Hill V, Yu Z, Restifo NP *et al* (2019) Neoantigen screening identifies broad TP53 mutant immunogenicity in patients with epithelial cancers. *J Clin Invest* **129**, 1109–1114.
10 Pitolli C, Wang Y, Mancini M, Shi Y, Melino G & Amelio I (2019) Do mutations turn p53 into an oncogene? *Int J Mol Sci* **20**, 6241.
11 Mantovani F, Collavin L & Del Sal G (2019) Mutant p53 as a guardian of the cancer cell. *Cell Death Differ* **26**, 199–212.
12 Levine AJ, Tomasini R, McKeon FD, Mak TW & Melino G (2011) The p53 family: guardians of maternal reproduction. *Nat Rev Mol Cell Biol* **12**, 259–265.
13 Melino S, Bellomaria A, Nepravishta R, Paci M & Melino G (2014) p63 threonine phosphorylation signals the interaction with the WW domain of the E3 ligase Itch. *Cell Cycle* **13**, 3207–3217.
14 Bellomaria A, Barbato G, Melino G, Paci M & Melino S (2010) Recognition of p63 by the E3 ligase ITCH: Effect of an ectodermal dysplasia mutant. *Cell Cycle* **9**, 3730–3739.
15 Lena AM, Cipollone R, Amelio I, Catani MV, Ramadan S, Browne G, Melino G & Candi E (2010) Skn-1a/Oct-11 and ΔNp63α exert antagonizing effects on human keratin expression. *Biochem Biophys Res Commun* **401**, 568–573. http://dx.doi.org/10.1016/j.bbrc.2010.09.102.
16 Nemajerova A, Amelio I, Gebel J, Dötsch V, Melino G & Moll UM (2018) Non-oncogenic roles of TAp73: from multiciliogenesis to metabolism. *Cell Death Differ* **25**, 144–153. http://dx.doi.org/10.1038/cdd.2017.178
17 Weiss RA (2020) A perspective on the early days of RAS research. *Cancer Metastasis Rev* **39**, 1023–1028.
18 Dao T, Pankov D, Scott A, Korontsvit T, Zakhaleva V, Xu Y, Xiang J, Yan S, de Morais Guerreiro MD, Veomett N *et al* (2015) Therapeutic bispecific T-cell engager antibody targeting the intracellular oncoprotein WT1. *Nat Biotechnol* **33**, 1079–1086. http://dx.doi.org/10.1038/nbt.3349
19 Fesnak AD, June CH & Levine BL (2016) Engineered T cells: the promise and challenges of cancer immunotherapy. *Nat Rev Cancer* **16**, 566–581.
20 Gonzalez-Sanchez P & DeNicola GM (2021) The microbiome(s) and cancer: know thy neighbor(s). *J Pathol* 5661.
21 Walker AR & Datta S (2019) Identification of city specific important bacterial signature for the MetaSUB CAMDA challenge microbiome data. *Biol Direct* **14**, 11.
22 Lennon JT & Locey KJ (2020) More support for Earth's massive microbiome. *Biol Direct* **15**, 5.

23 Zhu C, Miller M, Lusskin N, Mahlich Y, Wang Y, Zeng Z & Bromberg Y (2019) Fingerprinting cities: differentiating subway microbiome functionality. *Biol Direct* **14**. http://dx.doi.org/10.1186/s13062-019-0252-y
24 Melino S, Sabelli R & Paci M (2011) Allyl sulfur compounds and cellular detoxification system: effects and perspectives in cancer therapy. *Amino Acids* **41**, 103–112.
25 Aaes TL & Vandenabeele P (2021) The intrinsic immunogenic properties of cancer cell lines, immunogenic cell death, and how these influence host antitumor immune responses. *Cell Death Differ* **28**, 843–860.