ONCOIMMUNOLOGY
2022, VOL. 11, NO. 01, e2141978 (3 pages)
https://doi.org/10.1080/2162402X.2022.2141978

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COMMENTARY

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Bispecific T cell engagers kill resistant cells during KRAS-G12C blockade therapy

Xinxin Song, Zhuan Zhou, Rui Kang, and Daolin Tang

Department of Surgery, UT Southwestern Medical Center, Dallas, Texas, USA

ABSTRACT

The covalent KRAS-G12C inhibitors (G12Ci) are rapidly changing the treatment landscape for advanced non-small cell lung cancer, but drug resistance remains a clinical challenge. Two recent studies have developed bispecific T cell engagers that form a link between T cells and tumor cells to selectively eliminate G12Ci-resistant cells.

ARTICLE HISTORY
Received 22 October 2022
Revised 26 October 2022
Accepted 26 October 2022

KEYWORDS
KRAS-G12C; bispecific T cell engagers

Non-small cell lung cancer (NSCLC) is the predominant pathological type, accounting for 80% to 85% of human lung cancer cases. Certain gene mutations are associated with NSCLC. Of these, *KRAS* mutations (especially the hotspot *G12C* mutation) are present in about 30% of NSCLC and are more common in smokers.[1] Therefore, NSCLC appears to be the best tumor model for developing KRAS-G12C-targeted therapy, although colon and pancreatic tumors also contain a proportion of *G12C* mutations. Unfortunately, because the protein structure of KRAS-G12C lacks a significant binding pocket, direct KRAS-G12C inhibitors (G12Ci) have long been unavailable. After a 2013 study reported that the switch II pocket of KRAS-G12C protein can be bound by several small-molecule compounds (including 6H05, 2E07, and compounds 9/12),[2] pharmaceutical companies recently redesigned G12Ci based on this seminal finding, two of which (AMG510/sotorasib and MRTX849/adagrasib) are now used to treat patients with *KRAS-G12C*-mutated NSCLC.[3] Behind this great success is the emergence of drug resistance in some patients. Currently, preclinical studies offer strategies to overcome G12Ci resistance, most of which use chemotherapy or antibodies to target tumor growth-related signaling pathways.[4] A key unanswered question is how to design specific immunotherapies to enhance the effects of G12Ci?

In fact, several clinical trials are evaluating the combination of immune checkpoint inhibitors and G12Ci.[4] However, there is uncertainty about the specificity of this combination therapy. Recently, two independent groups established a new method for linking G12Ci-resistant cells and T cells using bispecific T-cell engagers (also known as BiTE) for specific clearance of drug-resistant cells through T cell-mediated cytotoxicity by releasing granzyme B and perforin (Figure 1).[5,6] BiTEs are fusion proteins, usually composed of two single-chain variable fragments (scFv) of different antibodies.[7] One of the scFvs binds to T cells through the CD3 receptor, and the other binds to tumor cells through tumor-specific molecules. Therefore, the specificity of BiTEs is mainly determined by tumor-specific molecules, namely neoantigens.

One of the advantages of BiTEs technology is that it bypasses traditional antigen presentation via major histocompatibility complex (MHC) class I molecules, although identification of antigenic peptides is necessary for the design of specific BiTEs.[7] Both groups identified a unique class of tumor-specific and KRAS-G12C mutant protein-dependent antigens that can be generated by chemical modification of G12Ci and undergo antigen processing, proteasomal degradation, peptide transport, presentation, and production of peptide-MHC class I complex.[5,6] Then, both groups designed specific BiTEs to recognize G12Ci-peptide-MHCI and T cells,[5,6] which are discussed below (Figure 1).

The first group from Zhang *et al* used a naive human B cell-derived Fab phage library to discover antibody fragments that specifically bind G12Ci (ARS1620)-modified peptides to N-terminal biotin immobilized on streptavidin-coated magnetic beads.[5] After 4 rounds of screening, the authors found that P1A4 has high affinity for the ARS1620-modified K5 peptide, either as a free peptide (58 nM) or when presented in A*02:01 MHC class I complex (62 nM). Next, they converted P1A4 to BiTE, linking the CD3-binding scFv with a short peptide linker. Functionally, this P1A4-based BiTE induced a cytolytic T-cell response that killed ARS1620-resistant cells *in vitro* and *in vivo*, albeit with some differences in the doses used. This group also designed a BiTE to identify P1B7 produced by sotorasib treatment. Therefore, although both are G12Ci, the key neoantigen peptides used for BiTE production are different.

The second group from Hattori *et al* developed a platform called "HapImmune", which created G12Ci-peptide conjugates as cancer neoantigens.[6] First, they used NetMHCpan[8] to predict peptides that mimic the bulky side chains of sotorasib-conjugated Cys12, which can be presented on HLA-A*03, -A*11, and -A*02. They bound sotorasib to these peptides and generated the MHC complexes using standard refolding procedures. Next, they determined the sequence profiles of antibodies against different sotorasib/MHC antigens by phage display combined with deep

CONTACT Daolin Tang daolin.tang@utsouthwestern.edu Department of Surgery, UT Southwestern Medical Center, Dallas, Texas 75390, USA

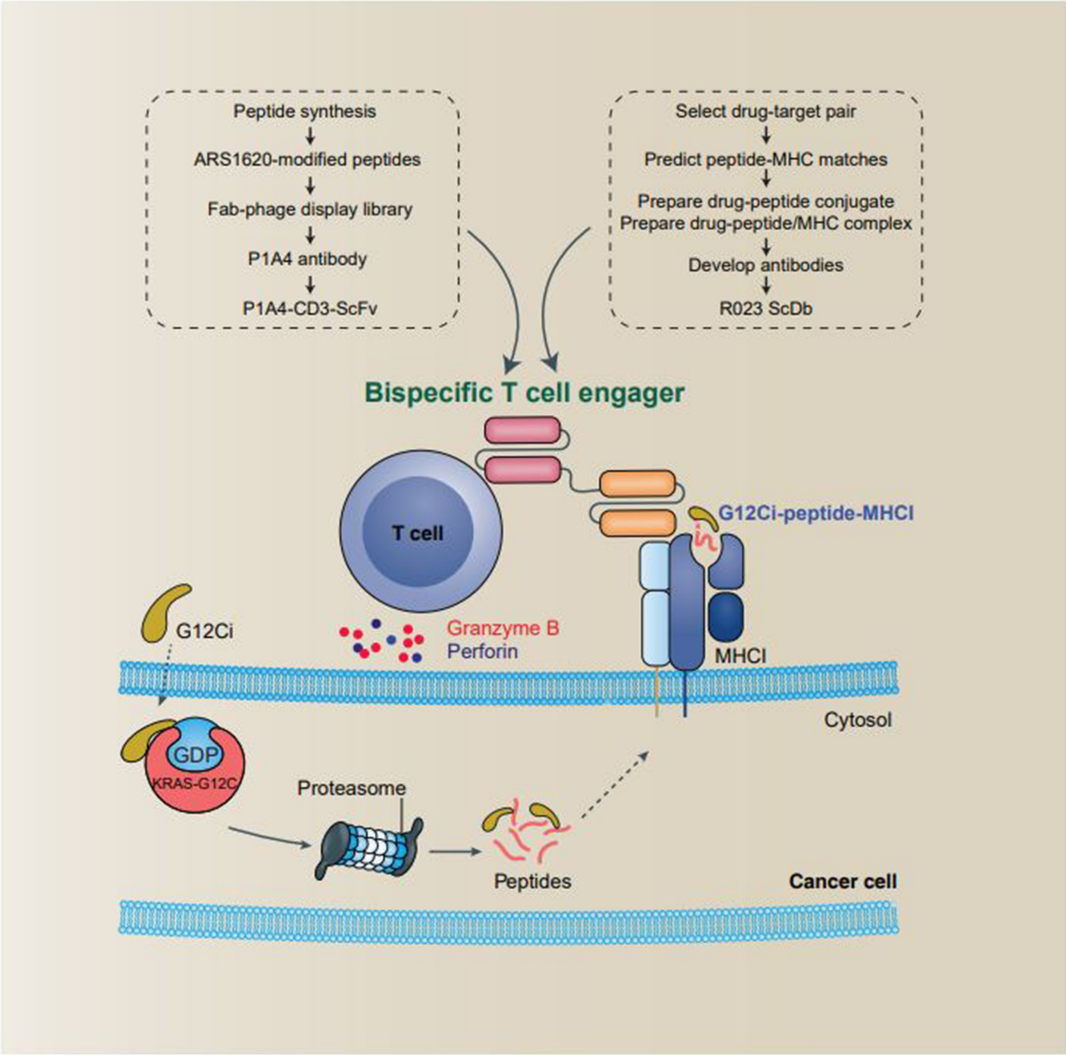

Figure 1. Bispecific T cell engagers designed to clear KRAS-G12C inhibitor-resistant cancer cells. G12C inhibitors (G12Ci) produce covalently modified peptides that undergo antigen processing, proteasomal degradation, peptide transport, presentation, and ultimately G12Ci-peptide-MHCI recognized by cytotoxic T cells. The top of the figure summarizes the bispecific T-cell engagers development strategies of two independent groups.

mutation scanning technology. They then designed a custom library and identified clone R023 with low nanomolar affinity for the target. Finally, they designed a BiTE (called R023 scDb; EC50 = 29 pM) by constructing a single-chain diabody (scDb) containing the HapImmune antibody for target cell recognition and a component that binds to CD3 on T lymphocytes. The R023 scDb has two properties: 1) the ability to bind to sotorasib-conjugated peptides in a manner not limited to a single HLA; 2) its specificity for inhibitor-peptide conjugates complexed with MHC is superior to free sotorasib. The HapImmune platform may be used to design BiTEs for clearance of other drug-resistant cells.

Taken together, these studies provide a novel immune antibody engineering approach for selective clearance of G12Ci-resistant cells, although additional preclinical and clinical trials are required to further evaluate its therapeutic efficacy and safety. These findings also reinforce the notion that neoantigens are generated not only in tumorigenesis but also in tumor therapy, which can be used to design various immunotherapies.[9] Further understanding of oncogene addiction and the immunogenicity of neoantigens in the tumor microenvironment is important for precision tumor therapy.[10] Engineering antibodies relies on knowledge of molecular targets and host immune responses. We also need to assess the risk of developing autoimmune disease with long-term use of therapeutic antibodies.

Disclosure statement

The authors declare no conflicts of interest or financial interests.

Funding

Research by D.T. and R.K. was supported by grants from the National Institutes of Health [R01CA160417, R01CA229275, and R01CA211070].

ORCID

Daolin Tang http://orcid.org/0000-0002-1903-6180

Data availability statement

All data that led to the conclusions in this manuscript have been referenced and all sources have been described.

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