Facts and Hopes in Immunotherapy Strategies Targeting Antigens derived from KRAS mutations

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Running Title: Mutant KRAS is an Immunological Target

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Abstract

In this commentary, we advance the notion that mutant KRAS (mKRAS) is an ideal tumor neoantigen that is amenable for targeting by the adaptive immune system. Recent progress highlights key advances on various fronts that validates mKRAS as a molecular target and supports further pursuit as an immunological target. Since mKRAS is an intracellular membrane localized protein and not normally expressed on the cell surface, we surmise that proteasome degradation will generate short peptides that bind to HLA class I (HLA-I) molecules in the endoplasmic reticulum for transport through the Golgi for display on the cell surface. T cell receptors (TCR)$\alpha\beta$ and antibodies have been isolated that specifically recognize mKRAS encoded epitope(s) or haptenated-mKRAS peptides in the context of HLA-I on tumor cells. Case reports using adoptive T cell therapy provide proof of principle that KRAS G12D can be successfully targeted by the immune system in cancer patients. Among the challenges facing investigators is the requirement of precision medicine to identify and match patients to available mKRAS peptide/HLA therapeutics and to increase the population coverage by targeting additional mKRAS epitopes. Ultimately, we envision mKRAS-directed immunotherapy as an effective treatment option for selected patients that will complement and perhaps, synergize with small molecule mKRAS inhibitors and targeted mKRAS degraders.

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Introduction

The RAS family of GTPases (KRAS, HRAS and NRAS) is essential for cell proliferation (1). It is estimated that ~20% of adult solid tumors harbor RAS genetic alterations which are primarily recurrent missense mutations – "hotspot mutations" - at codon Glycine 12 (G12), Glycine 13 (G13), or Glutamine 61 (Q61) (2). Importantly, these mutations impose a gain of function that dysregulate RAS activity leading to downstream activation of Raf/ERK and PI3K pathways. Various genetic and pharmacologic approaches (3) have validated KRAS as a clonal driver oncoprotein in pancreas adenocarcinoma (PAAD), colorectal carcinoma (COAD/READ) and lung adenocarcinoma (LUAD), which accounts for 37.7% (230,170 patients) of the estimated 609,820 cancer-related deaths per year in the US(4).

The most common KRAS mutations occur at G12 giving rise to G12D, G12V, G12C, and G12R variants which are frequent in PAAD, LUAD, and COAD/READ(2). Each of these amino acid substitutions may create a unique neoantigen encoded by the cancer cell when presented by HLA molecules to the adaptive immune system. Numerous immunological approaches have been taken to target mKRAS leading to a portfolio of constructs ranging from candidate vaccine antigens to TCRs to monoclonal antibodies (mAbs). The common theme is the requirement for mKRAS peptide/HLA complexes (p-HLA) as the primary target on the tumor cell surface. Here we aim to provide facts and hopes on the current state of this emerging topic in Immuno-Oncology.

Facts

Cancer Neoantigens

The relationship of tumor mutational burden (TMB) and clinical response to immune checkpoint blockade therapy (ICB) provided strong circumstantial evidence that tumor neoantigens were the targets of the patient's immune response. In cutaneous melanoma and tobacco-associated lung cancer, small cohort clinical trials with anti-CTLA-4 or anti-PD-1/PD-L1 mAbs provided the initial evidence for an association of high TMB (>10 mut/MB) with response rate (5,6). This relationship was later confirmed in larger multi-institutional trials and a consensus emerged that high TMB was associated with clinical benefit in many malignancies after treatment with ICB (7-10). Subsequent reports demonstrated the presence of neoantigen-specific T cells elicited by ICB which could recognize patient autologous tumor cells in vitro and thus, these T cells are likely critical effectors mediating tumor rejection. Likewise, microsatellite instable-high/mismatched-repair deficient patients had detectable T cell immunity directed against frameshift peptides implicating this class of antigens as the targets of the immune response elicited by ICB (11-14)

These reports were further substantiated by vaccine studies which provided evidence on the immunogenicity of tumor-encoded amino acid substituted peptides as neoantigens, with CD8+ and CD4+ T cells specific for these antigens detected pre- and post-vaccination in tumor and peripheral blood of cancer patients (15). Importantly, the majority of these neoantigens are

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encoded by unique (or private) passenger mutations displaying intratumor heterogeneity (ITH), defined as present in a fraction, not the totally, of tumor cells, and presumably playing a limited role in disease pathogenesis (16,17). This ITH is manifested as tumors composed of multiple populations or subclones, each expressing diversity in neoantigen composition. Studies have suggested that ITH may allow for the emergence of subclones and compromised neoantigen immunogenicity leading to immune evasion and resistance to immunotherapies(18-20). By contrast, low ITH even in high TMB, confers immunotherapy efficacy supporting the notion that clonal neoantigens may represent better cancer targets for therapeutic studies (18,19).

The rationale for targeting mKRAS

The frequency of mutant KRAS varies among human malignancies – ranging from 90% in pancreas cancer to 50% in colorectal cancer and 32% in lung adenocarcinoma to less than 5% in many rare tumors (2). Recent estimates suggest that 14% of new cancer diagnosis (~274,000 patients) in the US will harbor a KRAS mutation. In total, ~18.7% of new cancer patients (~362,000 patients) in the US will harbor a RAS mutation if one also includes NRAS and HRAS (2,4).

The history of RAS drug development underscores the essential challenges of target validation and highlights the need for a detailed understanding of the molecule of interest and converging signal transduction pathways (21-23). Recent studies reveal the frequent presence of somatic KRAS G12x variants in precursor lesions, pancreatic intraepithelial neoplasia, found in the pancreas of healthy adult (*bioRxiv* 2023 doi 10.1101/2023.01.27.525553). Current evidence supports the view that KRAS mutations represent an initiating clonal event which ultimately leads to invasive PAAD dependent on additional (loss of function) mutations or deletions in key genes such as *TP53*, *CDKN2A* and *SMAD4* (24). Similar observations have been made in lung adenocarcinoma related to mKRAS in tumor initiation (25). Compelling evidence supports the idea that sustained mKRAS signaling is required for tumorigenesis (26,27) although some studies conclude otherwise (28,29). Except for the endometrium, KRAS mutations are rarely detected in normal adult somatic tissues (30-32). Thus, mKRAS therapeutic agents rigorously assessed for on-target/off tumor toxicities with negligible reactivity to wild-type (WT) KRAs and human proteome enocoded peptides should provide unique opportunities for targeting cancer cells without risking normal tissue toxicities.

Oral KRAS G12C small molecule covalent inhibitors demonstrate significant single agent activity (37-43% ORR) in patients with lung cancer (33,34) which led to the regulatory approvals of sotorasib and adagrasib. The median progression-free survival (PFS) in these patient cohorts is 6.5-6.8 months; with rapid acquisition of drug resistance to single agent therapy (35), thus supporting combination therapy with a variety of compounds (36). New compounds targeting additional KRAS variants including pan-Ras inhibitors have been discovered and show initial promise (37). In the near term, investigators anticipate a steady stream of new small molecule KRAS inhibitors to enter the clinic (38).

The early years of mKRAS immunology

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In 1991, Jung and Schluesener first demonstrated that mKRAS (G12V) could be recognized by CD4+ T cells from normal healthy donors; moreover, T cell cultures established *in vitro* failed to react to WT KRAS peptide (39). The authors speculated that T cells specific for mKRAS would attack transformed cells in precancerous and malignant lesions and not harm healthy tissues. Peace and associates immunized inbred mice using synthetic mKRAS peptides in adjuvant to elicit CD4+ T cells which failed to react with WT KRAS peptide (40). Interestingly, several KRAS G12 variants, notably G12C, G12R, and G12V, were more immunogenic than others (G12D, G12S, and WT) when administered to C57BL6 and Balb/c mice. A series of studies from Schlom and co-workers investigated mKRAS peptides in mice and humans which further corroborated and extended the observation that mKRAS is immunogenic; thus, providing a strong foundation to justify a series clinical vaccination studies in solid tumor patients(41-43).

The first human vaccination trial against mKRAS was reported in 1995 by Gjertsen and coworkers. Five PAAD patients were genotyped and then immunized with mKRAS synthetic long peptide-pulsed PBMC with multiple booster doses (44). mKRAS specific CD4+ T cells were elicited in two of the 5 patients; in both cases, the T cells were specific for the immunizing mKRAS variant peptide (G12V and G12D) and failed to cross-react to WT KRAS. In this trial, clinical responses were not evident. Additional clinical trials performed at US National Institutes of Health and elsewhere confirmed the generation of mKRAS-specific T cell immunity in some patients but failed to demonstrate convincing clinical activity in solid tumor patients (45-48).

Proof of Concept: from TIL to TCR-T

A case report by Tran and colleagues at the NIH provided the initial evidence in humans that immune targeting of mKRAS could result in durable tumor regression (49). Infusion of an autologous tumor-infiltrating lymphocyte (TIL) product enriched in mKRAS G12D/HLA-C*08:02-specific CD8+ T cells resulted in a dramatic regression of numerous metastatic deposits, with confirmed partial clinical response, in a 50 year-old patient with metastatic COAD. Longer term persistence of multiple G12D-specific T cell clonotypes was observed and at 9 months post-infusion, a progressing solitary lung nodule was surgically excised. Molecular analysis of the lesion confirmed mKRAS G12D expression with selective loss of HLA-C*08:02, providing a molecular mechanism of tumor immune escape. Structural studies of the mKRAS G12D specific TCRs provides additional insights into the critical contact sites of the peptide:HLA complex (50).

A second case report from Leidner and colleagues using TCR-T cell adoptive therapy demonstrated clinical activity with regression (confirmed partial response) of multiple lung metastases in a patient with metastatic PAAD (51). In this instance, a combined infusion of autologous T cells engineered to express TCRs, cloned from the COAD patient in the initial report (49), directed against either nonamer or decamer G12D peptides-was administered after pre-conditioning with tocilizumab and cyclophosphamide followed by high dose IL-2. Long term persistence of the TCR-T cell products was documented, and the patient had an ongoing partial response at 6 months. A second PAAD patient receiving fludarabine and cyclophosphamide as pre-conditioning developed cytokine release syndrome and did not show tumor regression.

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Altogether, these proof-of-principle studies have garnered significant interest and prompted investigators to re-assess mKRAS as an immunological target.

The mKRAS Immuno-peptidome

The identification of antigenic peptides eluted from MHC class I (MHC-I) molecules of virally infected cells was a pivotal advance for the field of immunology (52,53). Landmark studies from the early-mid 1990's described sensitive MS/MS methods to identify both normal endogenous and tumor-specific peptides presented by HLA-I molecules in human cell lines (54,55). With increasing sensitivity of the instrumentation and improved bioinformatics, the field of immuno-peptidomics has matured and is currently in practice at many institutions worldwide (56).

A knowledge gap has prevailed regarding bone-fide (processed and HLA presented) candidate epitopes arising from amino acid substitution encoded by KRAS "hotspot" mutations. Our own work employed immuno-peptidomics to identify G12x epitopes presented by high-frequency HLA-I alleles (57). Using HLA mono-allelic cells lines transfected with mKRAS minigenes, 11 unique HLA-I restricted epitopes were identified, including both the nonamer and decamer peptides, (V)VVGAVGVGK restricted by HLA-A*11:01 as well as HLA-A*03:01. The G12D variant [(V)VVGADGVGK] is only detected as a decamer when presented by HLA-A*03:01 while both nonamer and decamer are presented by HLA-A*11:01. Decamer peptides targeting G12R [(V)VVGARGVGK] were also isolated in the context of HLA-A*03:01 and HLA-A*11:01. Novel epitopes [GARGVGKSAL] restricted to HLA-B*07:02 were also identified. Additional studies from other investigators have confirmed and extended this analysis to include over 39 HLA-I epitopes encoded by KRAS, NRAS, and HRAS variants (58-61) (**Table 1**). Interestingly, immuno-peptidomic studies from multiple groups have failed to identify mKRAS epitopes presented by HLA-A*02:01, the most frequent HLA-I allele in the US population.

mKRAS peptide /HLA class I therapeutics

Several strategies targeting mKRAS peptide/HLA-I complexes have been described in the last 5 years (**Figure 1**). First, our group and others have described TCRαβ directed at KRAS G12V [VVGAVGVGK and/or VVVGAVGVGK] in the context of HLA-A*11:01 (57,60). Additionally, we have also reported on TCRs directed at G12V [VVVGAVGVGK] in the context of HLA-A*03:01 and G12R [GARGVGKSAL] restricted by HLA-B*07:02(57). The functional avidities of these TCRs are in the nanomolar to picomolar range which enables recognition of human tumor cells with endogenous mKRAS and without reactivity to WT KRAS. Quantitative immuno-peptidomics performed using the KRAS G12V mutant COR-L23 lung adenocarcinoma cell line revealed a range of 8-78 G12V/A*11:01 or G12V/A*03:01 complexes per cell demonstrating recognition at physiologic levels of endogenous epitope on the cell surface. In a NSG xenograft lung metastases model, TCR-T cells directed at these epitopes promoted complete tumor rejection of CORL23 cell line and long-term survival (57). Selection of naturally-occurring TCRαβ candidates for clinical development will require safety profile evaluation as well as assessment

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of co-receptor (CD8/CD4) engagement, avidity and in vivo activity in humanized murine models (62).

TCRαβ may also be incorporated in a bispecific form linked to an anti-CD3 single-chain Fv (scFv), so called Immune Mobilizing Monoclonal T Cell receptors Against Cancer (ImmTACs). In this format, the TCR moiety provides the antigen specificity for the target complex, while the anti-CD3 crosslinks the TCR/CD3 complex of adjacent T cells for activation. Indeed, an ImmTAC presenting an affinity-enhanced human αβTCR targeting a KRAS G12D decamer peptide (VVVGA<u>D</u>GVGK) in the context of HLA-A*11:01 has been described (63). This agent displays potent re-directed killing activity against mKRAS G12D cancer cell lines *in vitro*; however, evidence for *in vivo* activity in a humanized mouse tumor model was not presented.

Antibodies have also been employed for targeting T cells to tumors. Antibody fragments (scFv) directed at a KRAS G12V decamer peptide (VVVGA<u>V</u>GVGK) in the context of HLA-A*03:01 are linked to anti-CD3 scFv in a Bispecific T cell Engager (BITE) format. These BITEs promote T cell activation in an antigen specific manner with no discernible reactivity against WT KRAS peptide. At sub-nanomolar concentrations using standard culture conditions, these bi-specific agents redirect T cells for lytic activity against KRAS G12V+ cancer cell lines in a HLA-restricted manner. In humanized xenograft models, these diabodies when administered by slow continuous release fail to promote tumor regression or prolong survival suggesting that further optimization is required (59,64).

As previously mentioned, small molecule covalent inhibitors of KRAS G12C have revolutionized molecular oncology by providing the first successful therapeutic strategy targeting mKRAS in solid tumors. Zhang and colleagues (65) hypothesized that ARS1620 (an investigational covalent G12C inhibitor) would create a haptenated G12C peptide that could be processed and presented by MHC-I on the tumor cell surface. Using a phage display library, a series of scFv were isolated that could specifically bind ARS1620-modified peptide as well as drug-treated human tumor cells harboring the KRAS G12C alteration. In a BITE format, a scFV (P1A4xCD3) construct mediated killing of cancer cell lines in an ARS1620-dependent and HLA independent manner. However, a significant limitations of study is the fact that these scFVs also recognize free drug preventing *in vivo* activity assessment. A study by Hattori et al. (66) employed a similar strategy to generate mAbs to sotorasib haptenated-mKRAS p/HLA-I complexes and create bispecific T cell engagers (R023) that could recognize drug exposed tumor cells which encode the KRAS G12C alteration. Although peptide presentation by HLA appears to be required for recognition by the R023 bispecific, recognition is not HLA restricted since these T cell engagers can recognize and kill drug-treated cancer cell lines expressing either HLA-A*02:01 or HLA-A*03:01. Although both studies demonstrate the possibility of developing novel bispecific T cell engagers directed against haptenated mKRAS peptides, formal studies are required to address the proteosomal degradation and presentation of haptenated peptides via the MHC-I pathway. Studies to determine whether R023 can recognize haptenated peptide on additional HLA alleles as well as characterization of additional novel hapten-specific reagents for targeting KRAS G12C are eagerly anticipated.

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Challenges and Hopes

Acknowledging the Challenges

We estimate that ~25% population coverage can be achieved with the mKRAS peptide/HLA-I strategies mentioned above in newly diagnosed PAAD, LUAD, and COAD patients expressing KRAS G12 variants (4,67) (**Figure 2**). However, the therapeutic utility of these targeted agents will depend not only on the prevalence of KRAS variants (in the context of HLA frequencies) but also on the development of rapid/cost-effective screening strategies to identify eligible patients. Currently, most patients are screened using standard HLA typing methods in a clinical lab with a <10 day turnaround time, while determination of mKRAS status is performed on tumor biopsy material by targeted NGS methods with a 10-21 day turnaround. Recent technological advances in genomics now permit rapid testing using liquid biopsy to assess KRAS mutation, including FDA approved liquid biopsy tests for G12C. Implementation of rapid testing platforms for routine qualification of patients for treatement with peptide/HLA-I therapeutics is viewed as a critical challenge that is necessary to identify target population and meet patient-physician expectations for timely initiation of cancer treatment.

Cancer cells under immune attack evade elimination by a variety of mechanisms (68,69). Alterations in HLA expression and/or antigen processing and presentation on cancer cells represent key mechanisms of resistance to immune therapies (70,71). A rapid evaluation of each patient's tumor may be required to characterize the immune defects related to HLA restriction as either reversible (soft) or irreversible (hard) as proposed by Aptsiauri and Garrido(70). The former may be correctable with cytokines and epigenetic modulators while the latter, although relatively uncommon, may not be fixable and thus, require alternative therapies not strictly dependent on p-HLA expression (e.g, NK cell therapies). An additional consideration is target-antigen density. In the steady state, the density of p-HLA complexes on the tumor cell surface appears to be in the range of 1-100 complexes per cell (72,73) although intra-patient heterogeneity of p-HLA expression is unknown. Finally, a mechanism of resistance to small molecule KRAS G12C inhibitors is the acquisition of additional mutations leading to loss of drug binding (35). Preclinical and clinical studies are necessary to determine whether haptenated G12C/HLA-I therapies may also lead to emergence of acquired resistance.

Need Help? - HLA class II restricted mKRAS neoantigens

Despite encouraging results from neoantigen mRNA vaccine trials and adoptive cell therapy trials, the precise mechanism(s) by which CD4+ T cells contribute to anti-cancer immunity remains ambiguous. A recent report, employing a KRAS G12V/HLA-DRB5*01:01 specific TCR, demonstrated that TCR-CD4+ T cells recognize transgene over-expressed KRAS G12V presented by antigen presenting cell lines (HLA-II+ lymphoblastoid cell lines) but failed to recognize HLA-II+/G12V+ lung carcinoma. These findings suggested that endogenously expressed G12V peptide is not presented through the MHC class II pathway and that recognition of these p-HLA-II complexes may be primarily mediated by cross presentation (74). Subsequently, the investigators provided experimental evidence that TCR-T CD4+ T cell

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generates effective therapeutic immunity by recruitment of tumor-specific CD8+ effector T cells which reject HLA-II negative tumors (75). Numerous TCRs directed at mKRAS p-HLA-II complexes have been described; however, no clinical trial data has been published so far (76-79). Based on the published evidence related to tumor-specific CD4+ T cells (80), and perhaps the added benefit of targeting multiple epitopes, the clinical testing of these HLA-II restricted TCRs conferring the "help" in combination with mKRAS /HLA-I TCRs, appears warranted.

When should we expect to see results?

While bygone vaccination trials against mKRAS solid tumors were safe and well-tolerated, little-no clinical activity was observed. This is not surprising as enrolled patients had refractory metastatic disease with limited projected survival. As vaccine platforms improve, newer adjuvants become available and more advanced immune monitoring methods are implemented (81), it is our expectation that the next generation of mKRAS cancer vaccines will advance to the clinic. Currently, several mKRAS cancer vaccine trials from industry and academia are open to accrual in the US and results are anticipated in the near term. As a note of caution, the inherent immunogenicity of mKRAS variants remains poorly defined at a population level given the paucity of data from the vaccine trials. It is anticipated that within the next 12 months several new clinical studies targeting mKRAS with adoptive T cell therapy will open in the US. Pre-clinical work related to bi-specific engagers and antibody drug conjugates is lagging and will likely take several years to enter the clinic. A combination of these agents with mKRAS small molecule inhibitors and targeted degraders (82) can be envisioned, allowing this oncogenic driver to be targeted at multiple levels.

The promise of targeting mKRAS – Closing Thoughts

In our view, targeting mKRAS will serve as a crucial test for cancer immunologists. We subscribe to the notion that targeting clonal driver neoantigens with the adaptive immune system is necessary and, in some instances, may be sufficient to promote durable remission in patients with metastatic solid tumors (83,84). Mutant KRAS fulfills most of the criteria (clonality, prevalence, selectivity and immunogenicity) used in the selection of cancer target antigens for $\alpha\beta$ T cells (85,86) and there is now sufficient evidence to believe that a rush will ensue to explore various therapeutic approaches (87). A series of mKRAS epitopes have been confirmed to be expressed by HLA-I molecules on the tumor cell surface further validating the immunological target. Multiple therapeutic strategies are anticipated including cancer vaccination, adoptive T cell transfer, antibody-drug conjugates and bi-specific molecules (88). Each platform represents an exciting area of investigation that should yield informative results and hopefully, multiple regulatory approvals.

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Figure Legends:

Figure 1 Immunotherapy strategies targeting antigens derived from KRAS mutations and mechanisms of action. Modalities based on TCR and antibody structures have been reported for targeting mKRAS peptide/HLA complexes (pHLA as shown). Each of these strategies relays on a different mechanism: Naturally-occurring (non-affinity enhanced) TCRab engineering endowing T cells with new targeting antigen specificity (top, left); ImmTACs engages pHLA in tumors thru an affinity-enhanced TCRab structure, while the anti-CD3 scFv portion engages T cells for lytic recognition (top, right). BITEs provide tumor recognition thru antibody scFv directed at pHLA complex (bottom, left) or at haptenated mKRAS peptide resulting from covalent small molecule inhibitor binding to peptide (bottom, right). The anti-CD3 scFv portion of BITE engages T cells and activates for lytic recognition. (Adapted from an image created with BioRender.com.)

Figure 2 Distribution of new diagnosed patient targetable by mKRAS peptide/HLA therapeutics. The newly diagnosed patient numbers per year are derived from Siegel et al (4), whereas the KRAS mutation frequencies are derived from AACR GENIE 9.0 Public Database (67) and the percentage of individuals expressing indicated HLA-I allele are based on Allele Frequency Net Database. From a total of 135,381 patients with mKRAS G12 variants, 33,070 will be eligible for potential therapies based on the strategies described in this review. This number may represent an underestimation as BITES targeting haptenated mKRAS peptide may cover a wider range of HLA-I alleles.

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Table 1: mKRAS Antigens identified by Proteomics and Immunological Assays.

HLA-I	mKRAS Mutation	Peptide	Peptide Length	Immuno-peptidomics	Immunogenicity	Reference
A*01:01	Q61H	ILDTAGHEEY	10	✓	✓	59,61
	Q61L	ILDTAGLEEY	10	✓	✓	59,61
	Q61R	ILDTAGREEY	10	✓	✓	59,61
A*03:01	G12C	VVVGACGVGK	10	✓		58
	G12D	VVVGADGVGK	10	✓		57, 58, 61
	G12D	VVGADGVGK	9	✓		61
	G12R	VVVGARGVGK	10	✓		58
	G12R	VVGARGVGK	9	✓		57
	G12V	VVVGAVGVGK	10	✓	✓	57, 58, 59
	G12V	VVGAVGVGK	10	✓		57, 58, 59
	G13D	VVVGAGDVGK	10	✓		61
	G13D	VVGAGDVGK	9	✓		61
A*11:01	G12C	VVVGACGVGK	10	✓	✓	57, 58
	G12D	VVVGADGVGK	10	✓	✓	57, 58, 60, 63
	G12D	VVGADGVGK	9	✓		57, 58
	G12R	VVVGARGVGK	10	✓		58
	G12R	VVGARGVGK	9	✓		57
	G12V	VVVGAVGVGK	10	✓	✓	57, 58, 60
	G12V	VVGAVGVGK	10	✓	✓	57, 58, 60
A*30:01	G12R	VVVGARGVGK	10	✓		58
	G12R	VVGARGVGK	9	✓		58
	G12V	VVVGAVGVGK	10	✓		58
	G12V	VVGAVGVGK	9	✓		58
A*68:01	G12C	VVVGACGVGK	10	✓		58
	G12D	VVVGADGVGK	10	✓		58
	G12R	VVVGARGVGK	10	✓		58
	G12V	VVVGAVGVGK	10	✓		58
	G12V	VVGAVGVGK	9	✓		58
B*07:02	G12D	GADGVGKSAL	10	✓		57, 58
	G12R	GARGVGKSAL	10	✓	✓	57, 58
C*01:02	G12V	AVGVGKSAL	9	✓		58
C*03:03	G12V	GAVGVGKSAL	10	✓		58
	G12V	GAVGVGKSA	9	✓		58
C*03:04	G12C	GACGVGKSA	9	✓		58
	G12D	GADGVGKSAL	10	✓		58
	G12V	GAVGVGKSAL	10	✓		58
	G12V	GAVGVGKSA	9	✓		58
C*08:02	G12D	GADGVGKSAL	10	✓	✓	49, 50, 51, 58
	G12D	GADGVGKSA	9	✓	✓	49, 50, 51, 58

Amino acid substitution in mutant KRAS peptide sequence is underlined.

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Figure 1

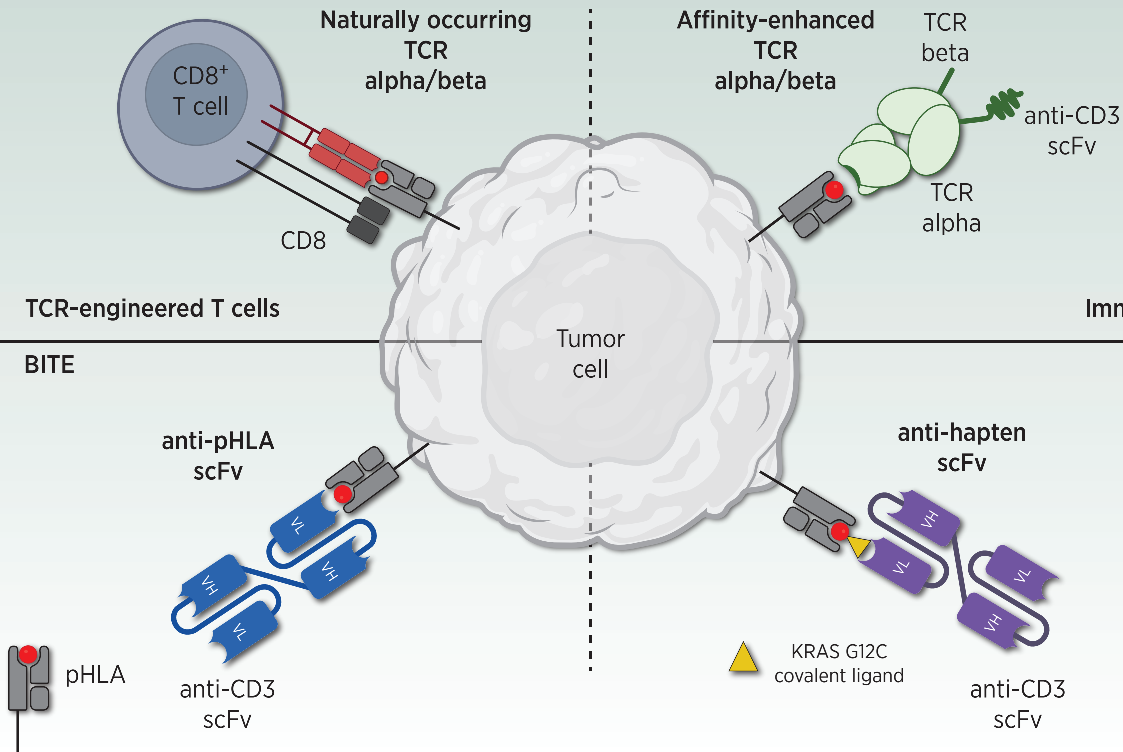

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Figure 2

				HLA			
	COAD	PAAD	LUAD	A*03:01	A*11:01	B*07:02	C*08:02
G12D	13,371	23,699	11,679		6,825		4,144
G12V	9,092	18,062	14,062	8,655	5,770		
G12C	2,888	705	32,414		5,041		
G12R	321	8,135	953			2,635	

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