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## Colorectal cancer vaccines: Tumor-associated antigens vs neoantigens

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### Abstract

Therapeutic options for the treatment of colorectal cancer (CRC) are diverse but still not always satisfying. Recent success of immune checkpoint inhibition treatment for the subgroup of CRC patients suffering from hypermutated tumors suggests a permanent role of immune therapy in the clinical management of CRC. Substantial improvement in treatment outcome could be achieved by development of efficient patient-individual CRC vaccination strategies. This mini-review summarizes the current knowledge on the two general classes of targets: tumor-associated antigens (TAAs) and tumor-specific antigens. TAAs like carcinoembryonic antigen and melanoma associated antigen are present in and shared by a subgroup of patients and a variety of clinical studies examined the efficacy of different TAA-derived peptide vaccines. Combinations of several TAAs as the next step and the development of personalized TAA-based peptide vaccines are discussed. Improvements of peptide-based vaccines achievable by adjuvants and immune-stimulatory chemotherapeutics are highlighted. Finally, we sum up clinical studies using tumor-specific antigens - in CRC almost exclusively neoantigens - which revealed promising results; particularly no severe adverse events were reported so far. Critical progress for clinical outcomes can be expected by individualizing neoantigen-based peptide vaccines and combining them with immune-stimulatory chemotherapeutics and immune checkpoint inhibitors. In light of these data and latest developments, truly personalized neoantigen-based peptide vaccines can be expected to fulfill modern precision medicine's requirements and will manifest as treatment pillar for routine clinical management of CRC.

**Key words:** Cancer vaccines; Colorectal neoplasm; Immunotherapy; Neoplasm antigen; Tumor-associated antigens; Tumor-specific antigens; Neoantigen(s)

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**Core tip:** Peptide vaccines are a promising tool for colorectal cancer (CRC) treatment. Direct comparison of tumor-associated antigens (TAAs) and neoantigens reveals clear superiority of the latter for several reasons. TAAs, albeit easier to identify and even shared by many patients, did not prove effective in clinical trials. Additionally, and due to their unspecificity, they frequently trigger severe adverse events. This risk is neglectable for tumor-specific neoantigens - thus compensating for the costly and laborious identification of such antigens expressed in individual patient tumors. Intelligent modern CRC vaccines will likely combine several or even many individual neoantigen-derived peptides with immuno-chemotherapy, adjuvants or further immuno-modulators.

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## INTRODUCTION

Therapy of colorectal carcinoma (CRC) has been improved over the years with advanced surgical and chemotherapeutic procedures but challenges in sight of efficiency and adverse effects must still be accomplished. Especially late stage CRC patients still have a relatively poor prognosis. Only recently, immunotherapy has reached general clinical acceptance with the break-through results of immune checkpoint inhibition for selected cancer types or subgroups - also for CRC.

One approach further improving this type of CRC therapy is the vaccination with peptides alone, peptide-expressing viruses, peptide-loaded antigen presenting cells or application of peptide-specific T cells. Historically, the development of such cancer vaccines started with peptides derived from tumor-associated antigens (TAAs).

## TAAs

TAAs are proteins that are significantly over-expressed in cancer compared to normal cells and are therefore also abundantly presented on the cancer cell's surface. Peptides of these TAAs bound to human leukocyte antigen (HLA) can be recognized by T cells initiating an anti-cancer immune response (Figure 1). Therefore, these TAAs have been used as target structures for the development of cancer vaccines (see Table 1 for an overview of CRC focused clinical studies).

## Carcinoembryonic antigen

One of the first TAAs ever identified was the carcino-embryonic antigen (CEA) which is also overexpressed in CRC<sup>[1]</sup>. In initial *in vitro* experiments, it could be proven that CEA-derived peptide-loaded dendritic cells (DCs) are able to induce CEA-specific cytotoxic T lymphocyte (CTL) activity<sup>[2]</sup>. However, a CEA-derived peptide with low avidity led to an inefficient immune response lacking activated CTLs<sup>[3]</sup>. The explanation for such inefficient T cell activation lies in the fact that TAAs like CEA are not really cancer specific but are also expressed by normal epithelial cells. Therefore, the organism must, for the most part, be tolerant to such TAAs in order to prevent autoimmunity.

Consequently, different approaches for modifying CEA vaccines were developed to overcome or weaken this immune tolerance. Using an altered peptide ligand of CEA with higher HLA binding affinity could efficiently activate specific CTLs *in vitro*<sup>[4]</sup>. Another way to enhance specific T cell activation was the development of DNA vaccines encoding the CEA-derived peptide(s) together with sequences for stimulating cytokines, adjuvants or supportive T helper cell epitopes. In murine models, this kind of vaccine showed higher T cell activation in comparison to peptide-only vaccines<sup>[5,6]</sup>. However, in clinical trials, the efficacy of CEA peptide vaccines was overall not satisfying, clinical response rate did not exceed 17 %<sup>[7-10]</sup>.

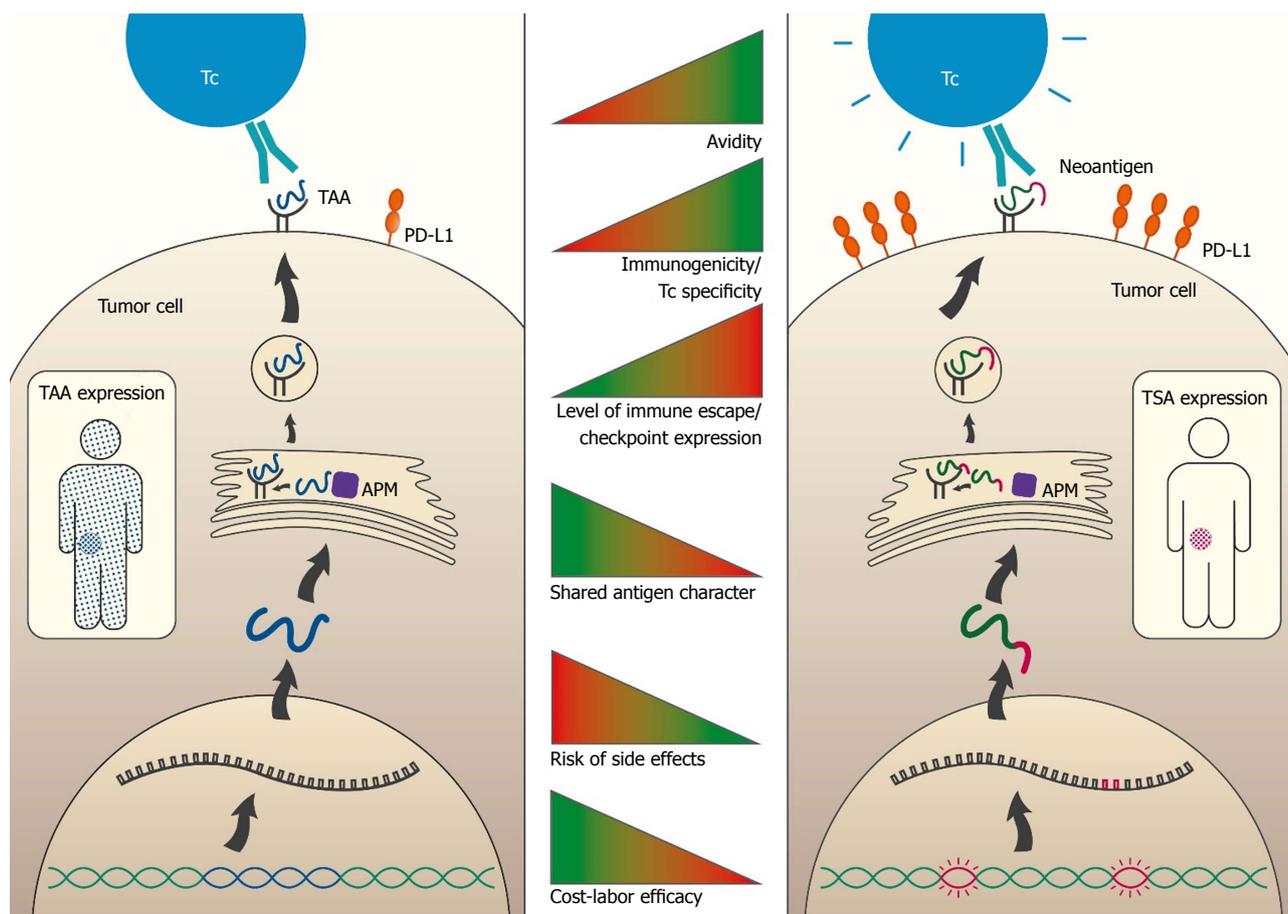
## Melanoma associated antigen

The melanoma associated antigen (MAGE), first discovered in melanomas, belongs to the group of cancer/testis antigens. This subgroup of TAAs is expressed only in testis and cancer cells. MAGE has subsequently been found to be expressed in the majority of adenocarcinomas. The rate of CRCs identified as being MAGE-positive strongly varied between different studies and MAGE variants: 14 % for MAGE-A<sup>[11]</sup>, 51 % for MAGE-A1-6<sup>[12]</sup> and 28 % for MAGE-A3<sup>[13]</sup>.

A clinical benefit by a MAGE-directed vaccination therapy could be shown in a case study by Takahashi *et al*<sup>[14]</sup>. They reported that a synthesized helper/killer-hybrid epitope long peptide of MAGE-A4 is able to induce an orchestrated CD4<sup>+</sup> and CD8<sup>+</sup> immune response leading to a slightly decreased tumor growth and resulting in stable disease. In a vaccination study investigating different TAAs, an increase in CEA-specific CTLs could be detected but clinical response was not observed<sup>[15]</sup>.

## Other TAAs

Progress in CRC vaccine development was made also with a variety of other TAAs. A peptide vaccination consisting of two different 9-mers derived from MUC-1 combined with CpG oligonucleotides and granulocyte macrophage colony-stimulating factor (GM-CSF) as adjuvants reduced tumor burden in a MUC1.Tg mouse model. In the prophylactic setting, even a complete



**Figure 1 Comparison of tumor-associated antigens and tumor-specific antigens properties.** The figure depicts properties and processing steps of antigens which are either tumor associated (TAA; blue; left side) or tumor specific (TSA; pink; right side). The course until antigen processing includes the following steps: transcription of genomic locus (TAA, blue) or mutation containing locus (TSA; pink), translation and RNA processing, protein degradation and MHC molecule loading and finally presentation of the antigen (TAA or TSA) on the cell surface embedded in MHC molecules. TAA-proteins are expressed to a high level in the tumor and to a low level in other organs and tissues (blue sprinkled patient). The neo-antigenic part of the TSA-protein is solely expressed in the tumor (pink sprinkled tumor). Recognition of the tumor cell by T cells (Tc, e.g., CTL) takes place via the T cell receptor (TCR green). The avidity is increased for TSAs (indicated by the “speedlines” on the right side of the T c). The tumor may counteract the immune recognition by expression of immune checkpoint molecules such as PD-L1 (orange). This occurs to a much higher extent in TSA bearing than in merely TAA bearing tumors. The middle panel indicates the degree of T cell avidity (first bar), extent of immunogenicity/T cell specificity (second bar), level of immune escape / checkpoint expression (third bar), shared antigen character (fourth bar), risk of side effects (fifth bar) and cost-labor efficacy (sixth bar) ranging from low (red) to high (green). TAA: Tumor-associated antigens.

protection against a syngeneic colon cancer cell line was achieved and attributed to the MUC 1-specific activation of the immune system<sup>[16]</sup>. However, these promising results could not be proven in clinical trials. Although an increase in anti MUC1 IgGs could be detected, neither cellular nor clinical response was observed<sup>[17-19]</sup>.

CRC patients treated with survivin-derived peptide-pulsed DCs showed an increased number of specific CTLs<sup>[20,21]</sup>. In a minority of patients, also a drop in level of tumor markers and even in total tumor volume was witnessed<sup>[21]</sup>.

Furthermore, success was achieved in CRC-focused studies on vaccination with peptides obtained from Wilms tumor 1 protein<sup>[22]</sup>, transmembrane 4 superfamily member 5 protein<sup>[23]</sup>, mitotic centromere associated kinesin<sup>[24]</sup> and epidermal growth factor receptor<sup>[25]</sup>.

Of note and in contrast to other tumor entities, NY-ESO-1 is not overexpressed in CRC<sup>[11,26,27]</sup> and has therefore not been exploited as a target for immuno-

therapy.

## COMBINATION OF TAAs

Single peptide vaccines often showed a significant immune response which was, however, not accompanied by a significant reduction of tumor burden. Thus, subsequent vaccination studies included more than one TAA-derived peptide to ameliorate clinical response.

After the identification of the ring finger protein 43 (RNF43) as a CTL-inducing peptide<sup>[28]</sup>, it was often investigated in combination with other peptides. At first Okuno *et al.*<sup>[29]</sup> combined chemotherapy with a RNF43 and a 34-kDa translocase of the outer mitochondrial membrane (TOMM34) peptide in a phase I clinical trial which resulted in 83 % stable disease and a mean survival time of 24 mo, but no reduction in tumor burden was observed. The efficiency of inducing specific CTLs by RNF43 and TOMM34 was also proven by additional

**Table 1 Clinical vaccination trials focused on colorectal cancer patients**

Target type	Target molecule	Vaccination strategy	Therapy	No. of CRC Patients	Clinical response	Ref.
TAA	CEA	Altered peptide loaded on DC		10/12	2 CR, 2 SD, 1 MR, 7 PD	[8]
TAA	CEA	CEA peptides pulsed DC		10	2 SD, 8 PD	[9]
TAA	CEA	CEA peptides pulsed DC		10	7 had CTL increase	[10]
TAA	MAGE	MAGE-A-pulsed DC		21	21 PD	[15]
TAA	MAGE	synthesized helper/killer-hybrid epitope long peptide (H/K-HELP) of MAGE-A4		1	SD	[14]
TAA	MUC1	MUC1-mannan fusion protein	Chemotherapy	18	2 SD, 16 PD	[17]
TAA	MUC1	100-amino acid synthetic MUC1 peptide with Poly-ICLC		39	20 responders (IgG), 19 non-responders	[18]
TAA	MUC1	irradiated allogeneic colorectal carcinoma cell lines with GM-CSF-producing bystander cell line (K562)		9	4 CR, 5 PD	[19]
TAA	Survivin	survivin-2B peptide		15	1 MR, 3 SD, 11 PD	[21]
TAA	WT1	HLA-A or HLA-DR restricted peptides on DCs	Chemotherapy	3	3 SD	[22]
TAA	RNF43, TOMM34	peptides, with Montanide ISA 51	Chemotherapy	21	16 SD	[29]
TAA	RNF43, TOMM34	HLA-A*2402-restricted peptides	Chemotherapy	22	13 CTL induction	[31]
TAA	RNF43, TOMM34	Peptides with Montanide ISA 51		24	6 SD, 18 PD	[30]
TAA + VEGFR	RNF43, TOMM34, FOXM1, MELK, HJURP, VEGFR-1, VEGFR-2	HLA-A2402-restricted peptides with Montanide ISA 51	Chemotherapy	30	3 PR, 15 SD, 12 PD	[32]
TAA + VEGFR	RNF43, TOMM34, KOC1, VEGFR-1, VEGFR-2	HLA-A*2402-restricted peptides with Montanide ISA 51		19	1 CR, 6 SD, 12 PD	[33]
PPV TAA	cypB, Ick, SART 1-3, ART4	2-4 HLA-A24-restricted Peptides matching to patient's pre-vaccination immune response with Montanide ISA 51		10	1 PR, 1 SD, 8 PD	[34]
PPV TAA	SART3, Lck, WHS, HNR, MRP3, PAP, EZH2, CEA, PSCA, UBE, Her2/neu, PSA, CypB	2-4 HLA-A24- or HLA-A2 restricted Peptides matching to patient's pre-vaccination immune response with Montanide ISA 51	Chemotherapy	7	1 SD, 6 PD	[35]
PPV TAA	SART2-3, Lck, MRP3, EIF4EBP, WHSC2, CypB, CEA, UBE, Her2/neu,	2-4 HLA-A24- or HLA-A2 restricted Peptides matching to patient's pre-vaccination immune response with Montanide ISA 51	Chemotherapy	14	3 MR, 3 SD, 8 PD	[36]
Neoantigen	AIM2(-1), HT001(-1), TAF1B(-1)	Frameshift peptides with Montanide ISA 51		22	16 immune response (CTL/IgG induction)	[52]
Neoantigen	KRAS	13-mer ras peptide with Detox adjuvant		10	1 SD, 2 cytotoxic activity	[62]
Neoantigen	KRAS	13-mer ras peptide with Detox adjuvant		7	4 remained with no evidence of disease	[64]
Neoantigen	KRAS	13-mer ras peptide with Il-2 or GM-CSF or both		38	4 SD, 34 PD	[66]

CRC: Colorectal cancer; CR: Complete response; CTL: Cytotoxic T lymphocyte; DC: Dendritic cell; GM-CSF: Granulocyte macrophage colony-stimulating factor; HLA: Human leukocyte antigen; MR: Minor response; PD: Progressive disease; PR: Partial response; SD: Stable disease; CEA: Carcinoembryonic antigen; TAA: Tumor-associated antigen.

studies<sup>[30,31]</sup>.

To further improve clinical response, Okuno *et al.*<sup>[32]</sup> tested a seven peptide vaccine containing peptides from RNF43, TOMM34, forkhead box M1, maternal embryonic leucine zipper-kinase, holliday junction recognizing-protein and vascular endothelial growth factor receptor 1 and 2 (VEGFR-1 and VEGFR-2). In 9 out of 30 vaccinated patients, a CTL response to all 7 peptides could be detected including two of the three partial responders of the study. Moreover, there was a correlation between

the number of vaccine peptide-specific CTL responses and overall survival<sup>[32]</sup>. Similar results were observed in a vaccination study with peptides from RNF43, TOMM34, VEGFR-1 and VEGFR-2 as well as insulin-like growth factor- II mRNA binding protein 3<sup>[33]</sup>.

## PERSONALIZED PEPTIDE VACCINES

To further enhance the efficiency of cancer vaccines, the next wave of trials focused on personalized pep-

tide vaccines. This personalization was achieved by measuring existing peptide-specific CTL precursors in the patients' blood, as well as screening for peptide-specific IgGs, followed by vaccination with CTL-reactive peptides.

In a phase I clinical trial, Sato *et al.*<sup>[34]</sup> treated 10 CRC patients with 2-4 matching peptides derived from cytochrom B, intestinal cell kinase, squamous cell carcinoma antigen recognized by T cells (SART)1-3 and ADP-ribosyltransferase 4. Peptide specific CTLs increased in 50 % of patients, peptide specific IgGs in 60 %. In half of the patients an elevated functional CTL activity was observed in cytotoxicity assays. In spite of this enhanced immune response, only two patients could clinically benefit from vaccination and had a partial response (reduction of metastasis' volume) and a stable disease, respectively.

In a subsequent study, the effect of a personalized peptide vaccine in combination with a 5-fluorouracil derivative was investigated<sup>[35]</sup>. After six vaccinations, six out of seven patients responded to at least one peptide with increased CTL and IgG levels. But only one patient showed stable disease. He responded to peptides derived from SART3, Tyrosine-protein kinase Lck and Wolf-Hirschhorn syndrome protein.

The combination of personalized peptide vaccination and chemotherapy resulting in clinical benefit was also proven in a further study. Hattori *et al.* vaccinated 14 metastatic CRC patients with up to four personal HLA-matched peptides. This was combined with a 5-fluorouracil based standard chemotherapy<sup>[36]</sup>. Although neither partial nor complete responses were obtained, three patients showed minor response, defined as a reduction in tumor size. Furthermore, three additional patients had stable disease. The strongest immune responses were induced by peptides derived from SART2/3, multidrug resistance-associated protein 3, Her2/neu, cytochrome B, ubiquitin-conjugating enzyme E2 and CEA.

Besides, it could be proven that the number of peptides with increased CTL responses after vaccination was also significantly predictive of favorable overall survival<sup>[37]</sup> similar to the correlation found in studies with combinations of TAA vaccines<sup>[32]</sup>.

## NEOANTIGENS - TRULY TUMOR-SPECIFIC ANTIGENS

The stepwise acquisition and accumulation of mutations has been generally recognized as major mechanism for cancer initiation and progression. It not only leads to enhanced or reduced expression of genes but also to the expression of sequence-modified proteins - the so-called neoantigens (see Figure 1). Hence, the probability of creating neoantigens is rising simply with the number of mutations present in a given cancer cell<sup>[38]</sup>. But the mutational burden and therefore the potential of expressing neoantigens varies clearly between different cancer entities. The highest somatic mutation frequency of around 10 mutations per megabase is found in cancers of the skin, lung and colorectum resulting in the expression

of approximately 150 nonsynonymous mutations within expressed genes<sup>[39]</sup>.

A hyper-mutational burden is caused by a deficiency in the mismatch repair system. This leads to replication errors especially in regions with repetitive nucleotides which are found in coding microsatellites. A mismatch repair deficient tumor shows microsatellite instability (MSI) with a huge variety of mutations. A similar or even still higher mutational burden can be caused by a mutation in the catalytic subunit of DNA polymerase epsilon (POLE) leading to hypermutation independent of MSI.

Because high neoantigen load of CRC has repeatedly been correlated with improved patient survival<sup>[40,41]</sup>, neoantigens have only recently been accepted as ideal targets for successful immunotherapy. In addition, as neoantigens are truly tumor-specific antigens (TSA) only presented by cancer cells but not by normal cells, the immune system can easily distinguish between malignant and healthy tissue - minimizing the risk of vaccination-induced severe adverse events (SAEs).

### TGFBR2 and other frameshift mutations

Studies focused on single nucleotide insertions or deletions in coding microsatellites and identified several proteins frequently affected by frameshift mutations in MSI<sup>high</sup> CRCs. Early a short form of the transforming growth factor beta receptor 2 (TGFβR II) was identified as such a frameshifted and therefore truncated protein with a role in tumor progression.

It could be proven that a 23-mer peptide derived from frameshift mutated TGFβR II is able to induce T cell proliferation predominantly in CD4<sup>+</sup> T (helper) cells<sup>[42]</sup>. This promising result was confirmed by results from a frameshift mutated TGFβR II-derived 9-mer peptide<sup>[43]</sup>. But in contrast to the former study, the induced T cells were predominantly CD8<sup>+</sup> and, more important, these activated T cells were able to lyse TGFβR II-mutated CRC cells in a HLA-restricted fashion.

TGFβR II-mutation-reactive T cells were also able to decrease tumor load in a mouse model and even significantly prolong survival<sup>[44]</sup> underlining the potential of frameshifted TGFβR II as immunotherapeutic target.

In further studies, it was proven that peptides derived from frameshifted caspase 5, mut-S homologue 3 and O-linked N-acetylglucosamine transferase gene are able to induce CTLs with cytotoxic activity against CRC cells<sup>[45-47]</sup>. In another approach, an antibody response directed against frameshifted homeobox protein CDX2 was detected in serum of a CRC patient<sup>[48]</sup>.

There are several further candidate genes frequently presenting with frameshift mutations in coding microsatellites: PTHL3, HT001, AC1, ACVR2, SLC23A1, BAX, TCF-4 and MSH3<sup>[49]</sup>. In addition peptides derived from frameshift-mutated MARCKS-1, MARCKS-2, TAF1B - 1, PCNXL2-2, TCF7L2-2, Baxα+1<sup>[50]</sup> as well as CREBBP, AIM2, EP300 and TTK<sup>[51]</sup> have been suggested to be taken into consideration for developing cancer vaccines for MSI<sup>high</sup> CRCs as auspicious experimental and bio-

informatic data proved their importance.

In a first clinical trial, 22 CRC patients received vaccines containing peptides of frameshifted AIM2, HT001 and TAF1B<sup>[52]</sup>. No vaccine-related SAEs were observed and the induced immune response was significant: all patients responded to at least one of the peptides.

### **KRAS: an example of point mutated genes**

In the process of cancer development mutations resulting in neoantigens can emerge in every coding region of the DNA. Neoantigens caused by KRAS are one example for point mutations in CRC. KRAS plays a major role in intracellular signaling cascades and is found mutated in more than 40 % of CRCs<sup>[53,54]</sup>. Most frequent mutations are located at codon 12 (G12D or G12V mutation) or 13 (G13D mutation) of the KRAS gene and result in single amino acid substitutions in the expressed protein<sup>[55,56]</sup>. Early approaches could prove that peptides derived from mutated KRAS can stimulate CTLs *in vitro*<sup>[57,58]</sup> as well as in pancreatic<sup>[59,60]</sup> and colorectal cancer<sup>[61]</sup>. A subsequent study also investigated the cytotoxic activity of mutated KRAS peptide-induced CTLs<sup>[62]</sup>. Only 2 of 10 CRC patients showed induction of peptide specific CD8<sup>+</sup> but in addition, these cytotoxic T cells were able to lyse HLA-A2-positive target cells incubated with the 10-mer mutant peptide. Similar results were obtained in a xenograft study, where peptide-specific T cells were able to delay the growth of KRAS mutant pancreatic tumors<sup>[63]</sup>.

In vaccination trials with peptides derived from mutated KRAS, clinical benefits for patients could be achieved. Although only two of seven CRC patients responded positive to a mutated KRAS peptide vaccination, four remained with no evidence of disease<sup>[64]</sup>. In a case report, one CRC patient was treated with activated T cells recognizing G12D KRAS<sup>[65]</sup>. After a single infusion, all seven lung metastases regressed for 9 mo until 1 metastatic lesion progressed.

To further enhance the immunological response in CRC patients, Rahma *et al.* combined the peptide vaccination of mutated KRAS with IL-2 or GM-CSF<sup>[66]</sup>. The strongest immune response could be detected in the group with GM-CSF as adjuvant; all CRC patients had an increase in interferon producing, specifically-activated T cells. Despite the high immune response rate, no patient showed clinical response and disease progressed in all cases. An increase in regulatory T cells, detectable in all CRC patients of this group, is a likely explanation for this negative result.

### **Other neoantigens**

Besides point and frameshift mutations there are further possibilities in creating neoantigens. Mutations at somatic splice sites as well as deregulated splicing factors can lead to alternative splice variants. A large-scale systematic investigation revealed that alternative splice variants in CRC are mainly caused by exon skipping, alternative promoter or terminator and intron retention<sup>[67]</sup>. A comparison between CRC and normal cells

demonstrated that there are alternative splice variants exclusively expressed by cancer cells<sup>[68,69]</sup>. That these altered peptides can be presented by HLA was already proven in a study focusing on melanoma<sup>[70]</sup>. Therefore, further investigation in this field of alternative splice variants could lead to an extended range of target structures for cancer vaccines.

Ditzel *et al.*<sup>[71]</sup> found a completely different mechanism of neoantigen generation. They proved that the apoptotic markers cytokeratin 8 and 18 are only proteolytically truncated in CRC tissue but not in normal colon epithelia. The cancer-associated forms of cytokeratin 8 and 18 are early apoptosis markers and recognized by a human antibody specific for a heterotypic conformational epitope. However, this and similar epitopes can hardly serve as target structures for T cell-specific immunotherapies.

## **CHALLENGES FOR DEVELOPING A CRC VACCINE**

### **Genetic configuration and target selection**

One important property for the development of a CRC vaccine is the genetic subtype of the patient. Around 15 % of CRCs are MSI<sup>high</sup> and provide therefore a variety of mutations leading to neoantigens which are, as described above, often derived from frameshift mutations. Another form of hypermutated CRC is caused by POLE mutations - but only 3 % of CRC patients fit into this category. MSI<sup>high</sup> or POLE mutations are responsible for a high mutational burden which is regularly correlated with increased lymphocyte infiltration into the tumors mirroring higher pre-vaccination antitumoral activity of the patients' immune system. Especially the intratumoral presence of CD8<sup>+</sup> CD45RO<sup>+</sup> T cells correlates with improved survival<sup>[72]</sup>. A CRC vaccine could further enhance or re-activate this anticancer activity and result in tumor reduction.

But also CRCs without MSI or POLE mutations show multiple genetic alterations. These immunogenic mutations, TAAs or neoantigens, need to be identified for vaccine development. Modern next-generation sequencing approaches open up the possibility of easy and fast sequencing but challenges in form of tumor heterogeneity are still to be accomplished. The whole mutational profile of a tumor is difficult to be depicted and for individualized vaccine development, it has to be considered that tumor sequencing can reveal only mutations of a subset of cells and at the time point of operation. The mutational profile of residual metastatic cells might differ<sup>[73,74]</sup>.

One promising approach is to focus on driver mutations that are responsible for maintenance of the transformed status and/or the progression of the individual tumor. Aiming at driver mutations is of advantage in comparison to passenger mutations, as the tumor cell's survival is dependent on these dysregulated gene products and therefore, immune escape by switching off or reverting such mutations is less likely to occur.

The concept of personalized peptide vaccination follows another approach. CRC patients are screened for the presence of CTLs and IgGs against known TAAs and neoantigens in addition to the determination of their HLA profile. Having the knowledge of the patients' HLA layout as well as the natural immunogenicity of the tumor, the vaccine can be adapted by choosing matching peptides for vaccination. This lowers the risk of SAEs by only enhancing the existing antitumoral immune response.

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## VACCINE DESIGN

When a promising target is found, the design of the vaccine starts. As mentioned before, suiting (better ideal) peptides as well as adjuvants and administration schedules need to be selected. Due to space restrictions, we focus here on peptide-based vaccination strategies and omit recombinant protein- and tumor lysate-based ones.

### **Single peptides, peptide-loaded antigen-presenting cells or ex vivo expanded T cells?**

Peptides used for vaccination can vary in length. When they directly bind into the peptide-binding groove of the HLA molecules, 8-10-mers (HLA-A/-B/-C) or 13-18-mers (HLA-DP/-DQ/-DR) are typically used. But the binding affinities of peptides to different HLA isoforms deviate. In addition, as the patient is restricted to its individual set of HLA alleles, the efficiency of a peptide vaccine is dependent on the selection of peptides and their best matching HLA. Furthermore, most of the current studies have only investigated peptides restricted to the most common HLA alleles HLA-A2 or HLA-A24, thereby limiting the number of patients benefitting from this therapy.

To circumvent HLA restriction, longer peptides (15-30-mer), so called synthetic long peptides, can be used as these peptides are internalized, processed and presented by antigen presenting cells. The risk of digestion by proteases is also decreased as long peptides form a tertiary structure and have therefore a longer half-life<sup>[75,76]</sup>.

Alternatively, using (autologous) cellular vaccine strategies, completely evades the problem of HLA-restriction and peptide degradation. They can be composed of antigen presenting cells (DCs, B cells or artificial antigen presenting cells<sup>[77]</sup>) which present the selected peptide(s) to both CD4<sup>+</sup> helper as well as CD8<sup>+</sup> effector T cells or the direct approach of applying T cells carrying tumor-antigen-specific T cell receptor(s). For the latter approach, patient specific T cells need to be isolated, expanded and stimulated *in vitro*. After this complex *ex vivo* procedure, a defined amount of functional T cells can be given back to the patient. By including also T helper cells or peptides activating T helper cells, a humoral immune response can be induced, too<sup>[14]</sup>.

### **Adjuvants**

To further enhance the strength of a vaccine, formulations typically include also adjuvants. Incomplete

Freund's adjuvant, alum, gold or nanoparticles as well as heat shock proteins and GM-CSF are such adjuvants which improve antigen stability, delivery, processing and presentation to T cells<sup>[78,79]</sup>. This is achieved by forming a depot at the injection site resulting in slow and prolonged peptide release and/or by induction of proliferation and migration of antigen presenting cells. GM-CSF, as well as TNF receptor ligands and TLR agonists like CpG oligonucleotides, additionally aim at enhancing costimulatory signals for T cell activation. Furthermore, cytokines like interferons or interleukins lead to enhanced immune response in different clinical trials<sup>[80,81]</sup>.

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## ADVERSE EVENTS

Cancer vaccines are characterized by a high safety and low toxicity profile. Different studies evaluated SAEs grade III/IV after cancer vaccine therapy in around 5500 cancer patients and observed a frequency of < 3%<sup>[82,83]</sup>. But especially by using vaccination approaches with TAAs, the possibility of damaging healthy tissue should not be neglected. As mentioned above, TAAs are not restricted to the tumor tissue but only expressed at higher levels compared to (some) normal cells.

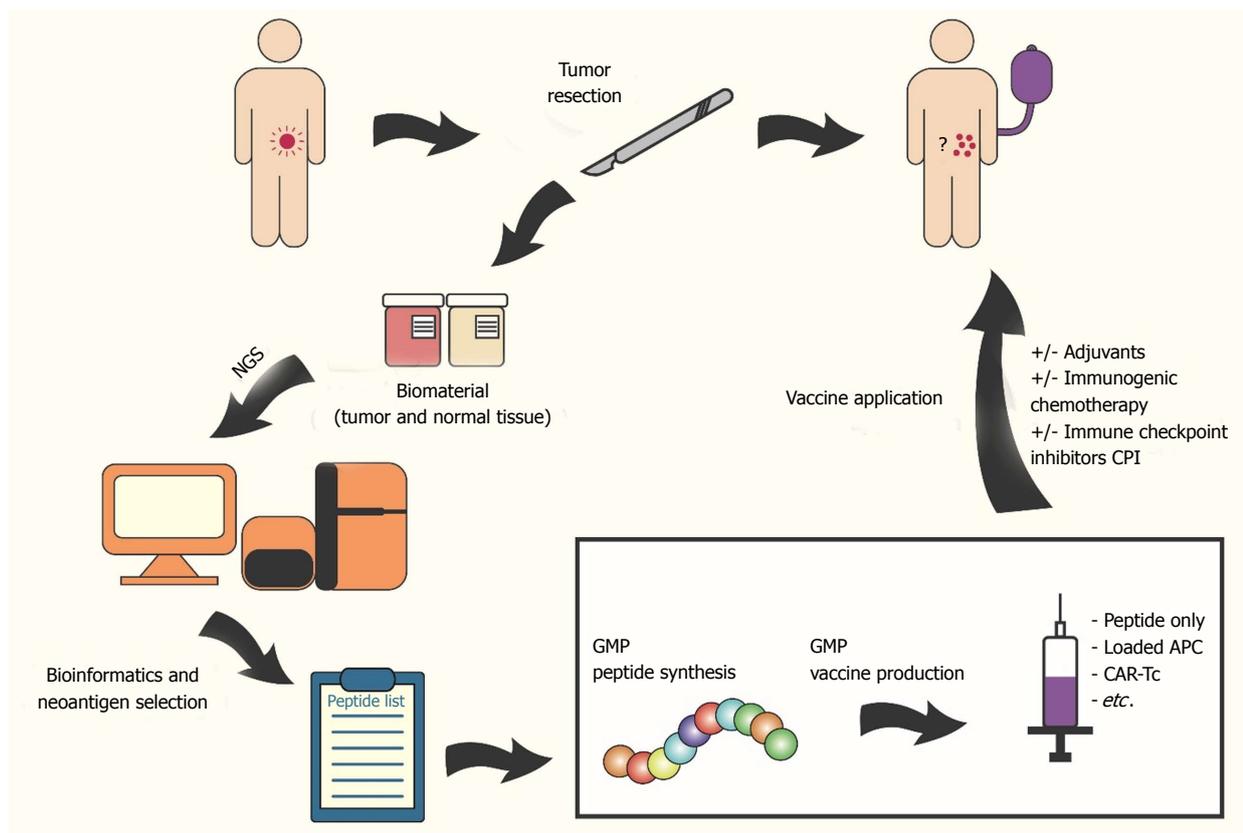
In a study with engineered anti-CEA T cells, the CEA levels decreased and even tumor regression was seen in one patient; but all treated patients experienced severe transient inflammatory colitis<sup>[84]</sup>. The treatment with autologous anti-MAGE-A3/A9/A12 engineered T cells led in another clinical trial to severe neurological toxicity in 3 out of 9 cancer patients<sup>[85]</sup>. Recognition of different MAGE-A proteins in normal human brain by engineered T cells caused even treatment related mortality in 2 patients. Similarly, the use of engineered T cells showing off-target effects by recognizing un-targeted proteins is associated with an increased risk of SAEs. Linette *et al.* used an affinity-enhanced T cell receptor against MAGE-A3 and the first two treated patients developed a cardiogenic shock and died within a few days<sup>[86]</sup>. Recognition of the striated muscle-specific protein titin by these T cells led to severe cardiogenic damage.

As neoantigens are not presented by healthy cells, the risk of SAEs is decreased by using neoantigen-targeting vaccines. To date, vaccine studies focusing on neoantigens in CRC patients observed no SAEs; only mild side effects that resolved spontaneously have been described (*e.g.*, injections site reactions, fever)<sup>[52,62,64,66]</sup>.

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## CANCER VACCINES: THE SOLUTION TO IMMUNE EVASION?

The tumor microenvironment is characterized by immunosuppressive signals leading to immune evasion of the tumor. An accumulation of regulatory T cells is responsible for the downregulation of other infiltrating and tumor attacking T cells in an antigen-dependent manner or by secretion of IL-10 or TGF- $\beta$ <sup>[87]</sup>. Furthermore, these signal molecules are able to suppress the maturation of



**Figure 2 Workflow: preparation of individualized vaccine (using neoantigen targets).** The figure shows the possible work flow for individualized cancer vaccination. The colorectal cancer patient (tumor in pink) undergoes tumor resection surgery and biomaterial (tumor (red container) and matching normal (beige container) tissue) is collected. Next generation sequencing and comparative bioinformatics analysis of these biomaterials reveal (tumor-specific) neoantigens and selected peptides are synthesized under GMP conditions. The vaccine consists of synthesized peptides, peptide-loaded antigen-presenting cells, *ex vivo* expanded T cells or chimeric antigen receptor T cells and can be combined with adjuvants, immunogenic chemotherapeutics and/or immune checkpoint inhibitors to further enhance vaccine efficacy. The patient will receive first vaccine shots ideally even before chemotherapeutic intervention. Residual tumor cells (in the colon or circulating as well as micrometastases in other organs) should be eliminated hereby. Exact vaccination scheme will depend on vaccine type, medical facility, *etc.*

DCs resulting in reduced antigen presentation<sup>[88]</sup>. Tumor-associated macrophages as well as myeloid suppressor cells are also present in the tumor microenvironment and act as immune suppressors<sup>[87,89]</sup>.

But in addition to creating an inhospitable environment for tumor attacking immune cells, tumor cells can also hide from immune cells by modifying their surface to escape recognition. More than 50 % of MSI<sup>high</sup> CRC patients' tumor harbor mutations that lower the functionality of HLA presentation of antigens<sup>[90]</sup>: mutations regarding regulation of HLA expression (*e.g.*, NLRC5 mutation<sup>[90]</sup>), peptide transport (*e.g.*, TAP1/2 and tapasin<sup>[90-92]</sup>) as well as HLA itself (*e.g.*, heavy chain<sup>[90]</sup> and B2M mutation<sup>[91-93]</sup>). To overcome this dissembling mechanism, the use of specific chemotherapeutics can be helpful, leading to an effective antitumor immune response by apoptosis (discussed below).

Furthermore, cancer cells take advantage of the control mechanism of nonsense mediated RNA-decay (NMRD). This system is responsible for degrading mRNA with premature stop codons and it has been suggested that this must inhibit the presentation of neoantigens<sup>[94]</sup>. However, it could be shown that only a part of the so far identified neoantigens are sensitive to NMRD<sup>[51]</sup>.

Last but not least, cancer cells can not only influence cells of the microenvironment to express immune checkpoint molecules but they frequently express such molecules themselves to downregulate T cell activity. Immune checkpoint inhibitors are a precious device in overcoming this tumor-induced immune suppression.

## IMMUNE CHECK POINT INHIBITORS

The field of studies exploring immune checkpoint inhibitors is growing. Targeted immune checkpoints like CTLA-4, PD-1, PD-L1 and LAG-3 are highly expressed in MSI<sup>high</sup> tumors, thereby creating an immune suppressive microenvironment<sup>[95]</sup>. Besides, T cells infiltrating in MSI<sup>high</sup> tumors frequently express PD-L1 making a PD-1 blocking antibody (*e.g.*, Pembrolizumab) a helpful instrument<sup>[96]</sup>. In clinical trials, almost 80 % of MSI high CRC patients benefitted from PD-1 blockade whereas microsatellite stable (MSS) CRC patients rarely did<sup>[96,97]</sup>. This can be explained by the difference in mutational burden of MSI<sup>high</sup> and MSS patients, as a correlation of mutational burden/number of neoantigens and clinical response could be proven already<sup>[98,99]</sup>. Moreover, this clinical observation suggests that a substantial part of

**Table 2** Current clinical vaccination studies including colorectal cancer patients

Target type	Target molecule	Vaccination strategy	Therapy	Number of patients	Trial identifier
TAA	CEA	alphavirus replicon (VRP) encoding CEA		12	NCT01890213
TAA	CEA	ETBX-011 ad-CEA, ALT-803 (IL-15)		3	NCT03127098
TAA	CEA	anti-CEA CAR-T cells		18	NCT03682744
TAA	CEA	anti-CEA CAR-T cells		5	NCT02850536
TAA	CEA	anti-CEA CAR-T cells, SIR-Sphere		8	NCT02416466
TAA	Her2	2 Her2 peptides in Montanide ISA 720		36	NCT01376505
TAA	Her2/neu	B-Cell and monocytes with HER2/neu antigen		9	NCT03425773
TAA	Brachyury, CEA, MUC1	ETBX-051; adenoviral brachyury vaccine, ETBX-061; adenoviral MUC1 vaccine, ETBX-011; adenoviral CEA vaccine		32	NCT03384316
TAA	7 cancer testis antigens	6 synthetic peptides in Montanide	Standard-of care maintenance	15	NCT03391232
Immune stimulation, TAA	MUC1	activated CIK and CD3-MUC1 bispecific antibody	cryotherapy	90	NCT03524274
TAA	HPV	DPX-E7		44	NCT02865135
TAA	hTERT	INO-1400 or INO-1401 alone or in combination with INO-9012		93	NCT02960594
TAA	MUC1	anti-MUC1 CAR-pNK cells		10	NCT02839954
TAA	MUC1	MUC1 peptide-poly-ICLC		110	NCT02134925
TAA	EpCAM	CAR T Cells targeting EpCAM		60	NCT03013712
Immune checkpoint, TAA	PD-1, p53	Pembrolizumab, modified vaccinia virus Ankara vaccine expressing p53		19	NCT02432963
Neoantigen		frameshift-derived neoantigen-loaded DC		25	NCT01885702
Neoantigen		personalized neoepitope yeast-based vaccine, YE-NEO-001		16	NCT03552718
Neoantigen		mRNA-based vaccine targeting neoantigens		64	NCT03480152
Neoantigen		ADXS-NEO (Advaxis NEO expressing personalized tumor antigens)		48	NCT03265080
Neoantigen	ras	anti-KRAS G12 V mTCR	Cyclophosphamide, Fludarabine, Aldesleukin	110	NCT03190941
Immune checkpoint	PD-L1	Avelumab, autologous dendritic cells		33	NCT03152565
Immune checkpoint	PD-1	Pembrolizumab, GVAX (allogeneic colon cancer GM-CSF secreting cells)	Cyclophosphamide	17	NCT02981524
Immune checkpoint	PD-L1	Atezolizumab, Imprime PGG (PAMP recognized by innate immune effector cells)	Regorafenib/ Isatuximab/ Bevacizumab	120	NCT03555149
Immune checkpoint	A2aR, A2bR	AB928 (A2aR and A2bR antagonist)	FOLFOX	98	NCT03720678
Immune checkpoint, TAA	PD-1, CEA, MUC-1	Nivolumab, MVA-BN-CV301 (modified vaccinia Ankara-Bavarian Nordic encoding CEA, MUC1, B7-1, ICAM-1 and LFA-3)	FOLFOX	78	NCT03547999
Immune checkpoint, TAA	PD-L1	Atezolizumab, RO7198457 (mRNA-based individualized, TAAs vaccine)		567	NCT03289962
Immune checkpoint, TAA	PD-L1, CEA	Avelumab + Ad-CEA	FOLFOX, Bevacizumab, Capecitabine	81	NCT03050814
Immune checkpoint, TAA, Immune stimulation	CEA, Her2/neu, Brachyury, MUC1, RAS, NK cells ICI	Aldoxorubicin, ETBX-011, ETBX-021, ETBX-051, ETBX-061, GI-4000, GI-6207, GI-6301, haNK, avelumab, HCl, ALT-803	Capecitabine, Cetuximab, Cyclophosphamide, Fluorouracil, Leucovorin, Nab-paclitaxel, Oxaliplatin, Regorafenib, SBRT, Trastuzumab	332	NCT03563157
Immune checkpoint, Mutated proteins	PD-1	Personalized peptides, Pembrolizumab		60	NCT02600949
Immune stimulation		GVAX (allogeneic colon cancer GM-CSF secreting cells)	Cyclophosphamide, SGI-110 (DNA Methyltransferase Inhibitor)	18	NCT01966289
Immune stimulation		GVAX (allogeneic colon cancer GM-CSF secreting cells)		15	NCT01952730

Immune stimulation		OncoVAX (non-dividing tumor cells)	Surgery	550	NCT02448173
Immune stimulation		Autologous or allogeneic immune stimulatory tumor cells		50	NCT00722228
Immune stimulation		autologous dendritic cells loaded with autologous tumour homogenate + IL-2		19	NCT02919644
Immune stimulation		autologous dendritic cells loaded with tumor antigens		58	NCT01348256
Immune stimulation		autologous dendritic cells loaded with tumor lysate antigens		30	NCT03214939
Oncolytic virus		GL-ONC1 oncolytic vaccinia virus, which disrupts nonessential genes and expression of the foreign gene expression		36	NCT02714374
Oncolytic virus, Immune checkpoint	PD-L1	Talimogene Laherpaprevac, Atezolizumab		36	NCT03256344

CEA: Carcinoembryonic antigen; GM-CSF: Granulocyte macrophage colony-stimulating factor; TAA: Tumor-associated antigen.

these antitumoral immune responses must be HLA-unrestricted, since more than the expected 50% of MSI<sup>high</sup> patients with functional HLA-presentation responded well. The interplay of adaptive (antitumoral) immune cells with cells of the innate arm of the immune system might partly explain this somewhat surprising finding. Consequently, this would also imply that modern neoantigen-specific vaccines (Figure 2) have a good chance to be beneficial for patients with hypermutated tumors despite the fact that HLA-presentation is corrupted due to immune escape phenomena.

Studies comparing infiltrating lymphocytes in MSS and MSI<sup>high</sup> CRC patients revealed, that the amount of infiltrating cells is clearly higher in MSI<sup>high</sup> tumors, but the correlation between infiltrating lymphocytes and overall survival is only in MSS patients significant<sup>[100,101]</sup>. Therefore, a combined immunotherapy with blocking immune checkpoints on the one hand and stimulating the immune system with a peptide vaccine on the other hand could help MSS as well as MSI<sup>high</sup> CRC patients. A first animal study demonstrated increased cytotoxicity rate, tumor suppression and survival with a DNA vaccine consisting of PD-1 fused with survivin and MUC-1 peptides<sup>[102]</sup>. Clinical trials investigating the effect of immune checkpoint inhibitors with other forms of immunotherapy in CRC patients are still ongoing (Table 2).

## CANCER VACCINES AND IMMUNOGENIC CELL DEATH

The dogma that chemotherapy is immunosuppressive has been disproved. Contrarily, it could be shown that selected chemotherapeutics are able to induce a special kind of cell death which improves tumor immune recognition. This so called immunogenic cell death is characterized by damage-associated molecular patterns on the surface of the tumor cell (calreticulin and heat shock proteins) which are "eat me" signals for immune cells and act as co-stimulators<sup>[103]</sup>. Inducers of immunogenic cell death are anthracyclines like doxorubicin<sup>[104]</sup>, DNA alkylating cyclophosphamide<sup>[105]</sup>

as well as the common platinum derivative for CRC treatment, oxaliplatin<sup>[106]</sup>. The studies combining TAAs with different chemotherapeutic agents also observed no immune suppressive effects but induction of specific immune responses<sup>[29,35,36]</sup>. Therefore, the combination of immunogenic cell death inducing chemotherapeutics and cancer-specific vaccination is an auspicious approach for future treatment of CRC patients (Figure 2).

Immunogenic cell death can also be induced by oncolytic viruses which preferentially infect cancer cells. *In vitro* studies revealed that viral treatment can lead to killing of CRC cells, especially tumor initiating cells or cancer stem cells<sup>[107,108]</sup>. First clinical studies with a combination of oncolytic viruses and immune checkpoint inhibitors in melanoma patients showed response rates up to 62 %<sup>[109-111]</sup>. These promising combinations are currently investigated in clinical trials also including CRC patients (Table 2).

## CONCLUSION

Cancer vaccines are a promising instrument for treatment of cancers. The development started with peptides derived from TAAs. These targets can be detected easily and are shared by many patients. Therefore, a variety of studies investigated the effect of TAA-derived peptide vaccines with mediocre results. Low immunogenicity, HLA restriction as well as increased risk of SAEs limit the efficiency and clinical usefulness of this vaccine type.

These problems can likely be solved with novel vaccination approaches focusing on TSAs, mainly neoantigens. They clearly differ from proteins of healthy cells and thus neither self-tolerance nor SAEs are likely to limit clinical application of TSA-based vaccines (see Figure 1). First *in vitro* and *in vivo* studies revealed promising results. It can be envisioned that these advantages will on the longer run compensate for the time and money intense identification of patient-individual neoantigens and peptide composition. Pure peptide vaccines, peptide-loaded antigen presenting cells or adoptively transferred T cells will be exploited.

To further enhance the effect of neoantigen vaccines, adjuvants will be included. These improve peptide stability and also act as immune stimulators. Besides, the combination of these new generation individual cancer vaccines with immune checkpoint inhibitors and/or immunogenic cell death-inducing chemotherapeutic agents is an utterly promising concept that will be extensively investigated in the near future. Such multifactorial approaches even have the potential to solve the difficulties in targeting MSS CRC. However, concepts to select the best-suited combinations of vaccines, adjuvants and chemotherapeutic on a patient-individual basis still have to be developed and - possibly even more ambitious - adapted to the clinical routine.

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## REFERENCES

- 1 **Gold P**, Freedman SO. Demonstration of tumor-specific antigens in human colonic carcinomata by immunological tolerance and absorption techniques. *J Exp Med* 1965; **121**: 439-462 [PMID: 14270243]
- 2 **Nair SK**, Hull S, Coleman D, Gilboa E, Lyerly HK, Morse MA. Induction of carcinoembryonic antigen (CEA)-specific cytotoxic T-lymphocyte responses in vitro using autologous dendritic cells loaded with CEA peptide or CEA RNA in patients with metastatic malignancies expressing CEA. *Int J Cancer* 1999; **82**: 121-124 [PMID: 10360830]
- 3 **Alves PM**, Viatte S, Fagerberg T, Michielin O, Bricard G, Bouzourene H, Vuilleumier H, Kruger T, Givel JC, Lévy F, Speiser DE, Cerottini JC, Romero P. Immunogenicity of the carcinoembryonic antigen derived peptide 694 in HLA-A2 healthy donors and colorectal carcinoma patients. *Cancer Immunol Immunother* 2007; **56**: 1795-1805 [PMID: 17447064 DOI: 10.1007/s00262-007-0323-2]
- 4 **Zaremba S**, Barzaga E, Zhu M, Soares N, Tsang KY, Schlom J. Identification of an enhancer agonist cytotoxic T lymphocyte peptide from human carcinoembryonic antigen. *Cancer Res* 1997; **57**: 4570-4577 [PMID: 9377571]
- 5 **Park JS**, Kim HS, Park HM, Kim CH, Kim TG. Efficient induction of anti-tumor immunity by a TAT-CEA fusion protein vaccine with poly(I:C) in a murine colorectal tumor model. *Vaccine* 2011; **29**: 8642-8648 [PMID: 21945963 DOI: 10.1016/j.vaccine.2011.09.052]
- 6 **Salucci V**, Mennuni C, Calvaruso F, Cerino R, Neuner P, Ciliberto G, La Monica N, Scarselli E. CD8+ T-cell tolerance can be broken by an adenoviral vaccine while CD4+ T-cell tolerance is broken by additional co-administration of a Toll-like receptor ligand. *Scand J Immunol* 2006; **63**: 35-41 [PMID: 16398699 DOI: 10.1111/j.1365-3083.2006.01706.x]
- 7 **Fong L**, Hou Y, Rivas A, Benike C, Yuen A, Fisher GA, Davis MM, Engleman EG. Altered peptide ligand vaccination with Flt3 ligand expanded dendritic cells for tumor immunotherapy. *Proc Natl Acad Sci USA* 2001; **98**: 8809-8814 [PMID: 11427731 DOI: 10.1073/pnas.141226398]
- 8 **Lesterhuis WJ**, de Vries IJ, Schuurhuis DH, Boullart AC, Jacobs JF, de Boer AJ, Scharenborg NM, Brouwer HM, van de Rakt MW, Figdor CG, Ruers TJ, Adema GJ, Punt CJ. Vaccination of colorectal cancer patients with CEA-loaded dendritic cells: antigen-specific T cell responses in DTH skin tests. *Ann Oncol* 2006; **17**: 974-980 [PMID: 16600979 DOI: 10.1093/annonc/mdl072]
- 9 **Liu KJ**, Wang CC, Chen LT, Cheng AL, Lin DT, Wu YC, Yu WL, Hung YM, Yang HY, Juang SH, Whang-Peng J. Generation of carcinoembryonic antigen (CEA)-specific T-cell responses in HLA-A\*0201 and HLA-A\*2402 late-stage colorectal cancer patients after vaccination with dendritic cells loaded with CEA peptides. *Clin Cancer Res* 2004; **10**: 2645-2651 [PMID: 15102666 DOI: 10.1158/1078-0432.CCR-03-0430]
- 10 **Okuno K**, Sugiura F, Itoh K, Yoshida K, Tsunoda T, Nakamura Y. Recent advances in active specific cancer vaccine treatment for colorectal cancer. *Curr Pharm Biotechnol* 2012; **13**: 1439-1445 [PMID: 22339221 DOI: 10.2174/138920112800784998]
- 11 **Kerkar SP**, Wang ZF, Lasota J, Park T, Patel K, Groh E, Rosenberg SA, Miettinen MM. MAGE-A is More Highly Expressed Than NY-ESO-1 in a Systematic Immunohistochemical Analysis of 3668 Cases. *J Immunother* 2016; **39**: 181-187 [PMID: 27070449 DOI: 10.1097/CJI.0000000000000119]
- 12 **Choi J**, Chang H. The expression of MAGE and SSSX, and correlation of COX2, VEGF, and survivin in colorectal cancer. *Anticancer Res* 2012; **32**: 559-564 [PMID: 22287745]
- 13 **Shantha Kumara HM**, Grieco MJ, Caballero OL, Su T, Ahmed A, Ritter E, Gnjatich S, Cekic V, Old LJ, Simpson AJ, Cordon-Cardo C, Whelan RL. MAGE-A3 is highly expressed in a subset of colorectal cancer patients. *Cancer Immunol* 2012; **12**: 16 [PMID: 23390371]
- 14 **Takahashi N**, Ohkuri T, Homma S, Ohtake J, Wakita D, Togashi Y, Kitamura H, Todo S, Nishimura T. First clinical trial of cancer vaccine therapy with artificially synthesized helper/killer-hybrid epitope long peptide of MAGE-A4 cancer antigen. *Cancer Sci* 2012; **103**: 150-153 [PMID: 22221328 DOI: 10.1111/j.1349-7006.2011.02106.x]
- 15 **Kavanagh B**, Ko A, Venook A, Margolin K, Zeh H, Lotze M, Schillinger B, Liu W, Lu Y, Mitsky P, Schilling M, Bercovici N, Loudovaris M, Guillermo R, Lee SM, Bender J, Mills B, Fong L. Vaccination of metastatic colorectal cancer patients with matured dendritic cells loaded with multiple major histocompatibility complex class I peptides. *J Immunother* 2007; **30**: 762-772 [PMID: 17893568 DOI: 10.1097/CJI.0b013e318133451c]
- 16 **Mukherjee P**, Pathangey LB, Bradley JB, Tinder TL, Basu GD, Akporiaye ET, Gendler SJ. MUC1-specific immune therapy generates a strong anti-tumor response in a MUC1-tolerant colon cancer model. *Vaccine* 2007; **25**: 1607-1618 [PMID: 17166639 DOI: 10.1016/j.vaccine.2006.11.007]
- 17 **Karanikas V**, Thynne G, Mitchell P, Ong CS, Gunawardana D, Blum R, Pearson J, Lodding J, Pietersz G, Broadbent R, Tait B, McKenzie IF. Mannan mucin-1 peptide immunization: influence of cyclophosphamide and the route of injection. *J Immunother* 2001; **24**: 172-183 [PMID: 11265775]
- 18 **Kimura T**, McKolanis JR, Dzubinski LA, Islam K, Potter DM, Salazar AM, Schoen RE, Finn OJ. MUC1 vaccine for individuals with advanced adenoma of the colon: a cancer immunoprevention feasibility study. *Cancer Prev Res (Phila)* 2013; **6**: 18-26 [PMID: 23248097 DOI: 10.1158/1940-6207.CAPR-12-0275]
- 19 **Zheng L**, Edil BH, Soares KC, El-Shami K, Uram JN, Judkins C, Zhang Z, Onners B, Laheru D, Pardoll D, Jaffee EM, Schulick RD. A safety and feasibility study of an allogeneic colon cancer cell vaccine administered with a granulocyte-macrophage colony stimulating factor-producing bystander cell line in patients with metastatic colorectal cancer. *Ann Surg Oncol* 2014; **21**: 3931-3937 [PMID: 24943235 DOI: 10.1245/s10434-014-3844-x]
- 20 **Idenoue S**, Hirohashi Y, Torigoe T, Sato Y, Tamura Y, Hariu H, Yamamoto M, Kurotaki T, Tsuruma T, Asanuma H, Kanaseki T, Ikeda H, Kashiwagi K, Okazaki M, Sasaki K, Sato T, Ohmura T, Hata F, Yamaguchi K, Hirata K, Sato N. A potent immunogenic general cancer vaccine that targets survivin, an inhibitor of apoptosis proteins. *Clin Cancer Res* 2005; **11**: 1474-1482 [PMID: 15746049 DOI: 10.1158/1078-0432.CCR-03-0817]
- 21 **Tsuruma T**, Hata F, Torigoe T, Furuhashi T, Idenoue S, Kurotaki T, Yamamoto M, Yagihashi A, Ohmura T, Yamaguchi K, Katsuramaki T, Yasoshima T, Sasaki K, Mizushima Y, Minamida H, Kimura H, Akiyama M, Hirohashi Y, Asanuma H, Tamura Y, Shimozawa K, Sato N, Hirata K. Phase I clinical study of anti-apoptosis protein, survivin-derived peptide vaccine therapy for patients with advanced or recurrent colorectal cancer. *J Transl Med* 2004; **2**: 19 [PMID: 15193151 DOI: 10.1186/1479-5876-2-19]
- 22 **Shimodaira S**, Sano K, Hirabayashi K, Koya T, Higuchi Y, Mizuno Y, Yamaoka N, Yuzawa M, Kobayashi T, Ito K, Koizumi T. Dendritic Cell-Based Adjuvant Vaccination Targeting Wilms' Tumor 1 in

- Patients with Advanced Colorectal Cancer. *Vaccines (Basel)* 2015; **3**: 1004-1018 [PMID: 26690485 DOI: 10.3390/vaccines3041004]
- 23 **Kwon S**, Kim YE, Park JA, Kim DS, Kwon HJ, Lee Y. Therapeutic effect of a TM4SF5-specific peptide vaccine against colon cancer in a mouse model. *BMB Rep* 2014; **47**: 215-220 [PMID: 24286311 DOI: 10.5483/BMBRep.2014.47.4.157]
- 24 **Kawamoto M**, Tanaka F, Mimori K, Inoue H, Kamohara Y, Mori M. Identification of HLA-A\*0201/-A\*2402-restricted CTL epitope-peptides derived from a novel cancer/testis antigen, MCAK, and induction of a specific antitumor immune response. *Oncol Rep* 2011; **25**: 469-476 [PMID: 21165574 DOI: 10.3892/or.2010.1101]
- 25 **Foy KC**, Wygle RM, Miller MJ, Overholser JP, Bekaii-Saab T, Kaumaya PT. Peptide vaccines and peptidomimetics of EGFR (HER-1) ligand binding domain inhibit cancer cell growth in vitro and in vivo. *J Immunol* 2013; **191**: 217-227 [PMID: 23698748 DOI: 10.4049/jimmunol.1300231]
- 26 **Jungbluth AA**, Chen YT, Stockert E, Busam KJ, Kolb D, Iversen K, Coplan K, Williamson B, Altorki N, Old LJ. Immunohistochemical analysis of NY-ESO-1 antigen expression in normal and malignant human tissues. *Int J Cancer* 2001; **92**: 856-860 [PMID: 11351307 DOI: 10.1002/ijc.1282]
- 27 **Stockert E**, Jäger E, Chen YT, Scanlan MJ, Gout I, Karbach J, Arand M, Knuth A, Old LJ. A survey of the humoral immune response of cancer patients to a panel of human tumor antigens. *J Exp Med* 1998; **187**: 1349-1354 [PMID: 9547346]
- 28 **Uchida N**, Tsunoda T, Wada S, Furukawa Y, Nakamura Y, Tahara H. Ring finger protein 43 as a new target for cancer immunotherapy. *Clin Cancer Res* 2004; **10**: 8577-8586 [PMID: 15623641 DOI: 10.1158/1078-0432.CCR-04-0104]
- 29 **Okuno K**, Sugiura F, Hida JI, Tokoro T, Ishimaru E, Sukegawa Y, Ueda K. Phase I clinical trial of a novel peptide vaccine in combination with UFT/LV for metastatic colorectal cancer. *Exp Ther Med* 2011; **2**: 73-79 [PMID: 22977472 DOI: 10.3892/etm.2010.182]
- 30 **Kawamura J**, Sugiura F, Sukegawa Y, Yoshioka Y, Hida JI, Hazama S, Okuno K. Multicenter, phase II clinical trial of peptide vaccination with oral chemotherapy following curative resection for stage III colorectal cancer. *Oncol Lett* 2018; **15**: 4241-4247 [PMID: 29541190 DOI: 10.3892/ol.2018.7905]
- 31 **Taniguchi H**, Iwasa S, Yamazaki K, Yoshino T, Kiryu C, Naka Y, Liew EL, Sakata Y. Phase I study of OCV-C02, a peptide vaccine consisting of two peptide epitopes for refractory metastatic colorectal cancer. *Cancer Sci* 2017; **108**: 1013-1021 [PMID: 28266765 DOI: 10.1111/cas.13227]
- 32 **Okuno K**, Sugiura F, Inoue K, Sukegawa Y. Clinical trial of a 7-peptide cocktail vaccine with oral chemotherapy for patients with metastatic colorectal cancer. *Anticancer Res* 2014; **34**: 3045-3052 [PMID: 24922671]
- 33 **Hazama S**, Nakamura Y, Takenouchi H, Suzuki N, Tsunedomi R, Inoue Y, Tokuhisa Y, Iizuka N, Yoshino S, Takeda K, Shinozaki H, Kamiya A, Furukawa H, Oka M. A phase I study of combination vaccine treatment of five therapeutic epitope-peptides for metastatic colorectal cancer; safety, immunological response, and clinical outcome. *J Transl Med* 2014; **12**: 63 [PMID: 24612787 DOI: 10.1186/1479-5876-12-63]
- 34 **Sato Y**, Maeda Y, Shomura H, Sasatomi T, Takahashi M, Une Y, Kondo M, Shinohara T, Hida N, Katagiri K, Sato K, Sato M, Yamada A, Yamana H, Harada M, Itoh K, Todo S. A phase I trial of cytotoxic T-lymphocyte precursor-oriented peptide vaccines for colorectal carcinoma patients. *Br J Cancer* 2004; **90**: 1334-1342 [PMID: 15054451 DOI: 10.1038/sj.bjc.6601711]
- 35 **Sato Y**, Fujiwara T, Mine T, Shomura H, Homma S, Maeda Y, Tokunaga N, Ikeda Y, Ishihara Y, Yamada A, Tanaka N, Itoh K, Harada M, Todo S. Immunological evaluation of personalized peptide vaccination in combination with a 5-fluorouracil derivative (TS-1) for advanced gastric or colorectal carcinoma patients. *Cancer Sci* 2007; **98**: 1113-1119 [PMID: 17459063 DOI: 10.1111/j.1349-7006.2007.00498.x]
- 36 **Hattori T**, Mine T, Komatsu N, Yamada A, Itoh K, Shiozaki H, Okuno K. Immunological evaluation of personalized peptide vaccination in combination with UFT and UZEL for metastatic colorectal carcinoma patients. *Cancer Immunol Immunother* 2009; **58**: 1843-1852 [PMID: 19396597 DOI: 10.1007/s00262-009-0695-6]
- 37 **Kibe S**, Yutani S, Motoyama S, Nomura T, Tanaka N, Kawahara A, Yamaguchi T, Matsueda S, Komatsu N, Miura M, Hinai Y, Hattori S, Yamada A, Kage M, Itoh K, Akagi Y, Sasada T. Phase II study of personalized peptide vaccination for previously treated advanced colorectal cancer. *Cancer Immunol Res* 2014; **2**: 1154-1162 [PMID: 25351849 DOI: 10.1158/2326-6066.CIR-14-0035]
- 38 **Rooney MS**, Shukla SA, Wu CJ, Getz G, Hacohen N. Molecular and genetic properties of tumors associated with local immune cytolytic activity. *Cell* 2015; **160**: 48-61 [PMID: 25594174 DOI: 10.1016/j.cell.2014.12.033]
- 39 **Schumacher TN**, Schreiber RD. Neoantigens in cancer immunotherapy. *Science* 2015; **348**: 69-74 [PMID: 25838375 DOI: 10.1126/science.aaa4971]
- 40 **Brown SD**, Warren RL, Gibb EA, Martin SD, Spinelli JJ, Nelson BH, Holt RA. Neo-antigens predicted by tumor genome meta-analysis correlate with increased patient survival. *Genome Res* 2014; **24**: 743-750 [PMID: 24782321 DOI: 10.1101/gr.165985.113]
- 41 **Giannakis M**, Mu XJ, Shukla SA, Qian ZR, Cohen O, Nishihara R, Bahl S, Cao Y, Amin-Mansour A, Yamauchi M, Sukawa Y, Stewart C, Rosenberg M, Mima K, Inamura K, Noshko K, Nowak JA, Lawrence MS, Giovannucci EL, Chan AT, Ng K, Meyerhardt JA, Van Allen EM, Getz G, Gabriel SB, Lander ES, Wu CJ, Fuchs CS, Ogino S, Garraway LA. Genomic Correlates of Immune-Cell Infiltrates in Colorectal Carcinoma. *Cell Rep* 2016; **15**: 857-865 [PMID: 27149842 DOI: 10.1016/j.celrep.2016.03.075]
- 42 **Saeterdal I**, Bjørheim J, Lislserud K, Gjertsen MK, Bukholm IK, Olsen OC, Nesland JM, Eriksen JA, Møller M, Lindblom A, Gaudernack G. Frameshift-mutation-derived peptides as tumor-specific antigens in inherited and spontaneous colorectal cancer. *Proc Natl Acad Sci USA* 2001; **98**: 13255-13260 [PMID: 11687624 DOI: 10.1073/pnas.231326898]
- 43 **Linnebacher M**, Gebert J, Rudy W, Woerner S, Yuan YP, Bork P, von Knebel Doeberitz M. Frameshift peptide-derived T-cell epitopes: a source of novel tumor-specific antigens. *Int J Cancer* 2001; **93**: 6-11 [PMID: 11391614 DOI: 10.1002/ijc.1298]
- 44 **Inderberg EM**, Wälchli S, Myhre MR, Trachsel S, Almäsbaek H, Kvalheim G, Gaudernack G. T cell therapy targeting a public neoantigen in microsatellite instable colon cancer reduces *in vivo* tumor growth. *Oncoimmunology* 2017; **6**: e1302631 [PMID: 28507809 DOI: 10.1080/2162402X.2017.1302631]
- 45 **Garbe Y**, Maletzki C, Linnebacher M. An MSI tumor specific frameshift mutation in a coding microsatellite of MSH3 encodes for HLA-A0201-restricted CD8+ cytotoxic T cell epitopes. *PLoS One* 2011; **6**: e26517 [PMID: 22110587 DOI: 10.1371/journal.pone.0026517]
- 46 **Ripberger E**, Linnebacher M, Schwitalle Y, Gebert J, von Knebel Doeberitz M. Identification of an HLA-A0201-restricted CTL epitope generated by a tumor-specific frameshift mutation in a coding microsatellite of the OGT gene. *J Clin Immunol* 2003; **23**: 415-423 [PMID: 14601650]
- 47 **Schwitalle Y**, Linnebacher M, Ripberger E, Gebert J, von Knebel Doeberitz M. Immunogenic peptides generated by frameshift mutations in DNA mismatch repair-deficient cancer cells. *Cancer Immunol* 2004; **4**: 14 [PMID: 15563124]
- 48 **Ishikawa T**, Fujita T, Suzuki Y, Okabe S, Yuasa Y, Iwai T, Kawakami Y. Tumor-specific immunological recognition of frameshift-mutated peptides in colon cancer with microsatellite instability. *Cancer Res* 2003; **63**: 5564-5572 [PMID: 14500396]
- 49 **Woerner SM**, Benner A, Sutter C, Schiller M, Yuan YP, Keller G, Bork P, Doeberitz M, Gebert JF. Pathogenesis of DNA repair-deficient cancers: a statistical meta-analysis of putative Real Common Target genes. *Oncogene* 2003; **22**: 2226-2235 [PMID: 12700659 DOI: 10.1038/sj.onc.1206421]
- 50 **Speetjens FM**, Lauwen MM, Franken KL, Janssen-van Rhijn CM, van Duikeren S, Bres SA, van de Velde CJ, Melief CJ, Kuppen PJ, van der Burg SH, Morreau H, Offringa R. Prediction of the immunogenic potential of frameshift-mutated antigens in microsatellite instable cancer. *Int J Cancer* 2008; **123**: 838-845 [PMID: 18506693 DOI:

- 10.1002/ijc.23570]
- 51 **Williams DS**, Bird MJ, Jorissen RN, Yu YL, Walker F, Zhang HH, Nice EC, Burgess AW. Nonsense mediated decay resistant mutations are a source of expressed mutant proteins in colon cancer cell lines with microsatellite instability. *PLoS One* 2010; **5**: e16012 [PMID: 21209843 DOI: 10.1371/journal.pone.0016012]
  - 52 **Kloor M**, Reuschenbach M, Karbach J, Rafiyan M, Al-Batran S-E, Pauligk C, Jaeger E, Knebel Doeberitz M von. Vaccination of MSI-H colorectal cancer patients with frameshift peptide antigens: A phase I/IIa clinical trial. *J Clin Oncol* 2015; **33**: 3020
  - 53 **Karapetis CS**, Khambata-Ford S, Jonker DJ, O'Callaghan CJ, Tu D, Tebbutt NC, Simes RJ, Chalchal H, Shapiro JD, Robitaille S, Price TJ, Shepherd L, Au HJ, Langer C, Moore MJ, Zalberg JR. K-ras mutations and benefit from cetuximab in advanced colorectal cancer. *N Engl J Med* 2008; **359**: 1757-1765 [PMID: 18946061 DOI: 10.1056/NEJMoa0804385]
  - 54 **Vaughn CP**, Zobel SD, Furtado LV, Baker CL, Samowitz WS. Frequency of KRAS, BRAF, and NRAS mutations in colorectal cancer. *Genes Chromosomes Cancer* 2011; **50**: 307-312 [PMID: 21305640 DOI: 10.1002/gcc.20854]
  - 55 **Andreyev HJ**, Norman AR, Cunningham D, Oates JR, Clarke PA. Kirsten ras mutations in patients with colorectal cancer: the multicenter "RASCAL" study. *J Natl Cancer Inst* 1998; **90**: 675-684 [PMID: 9586664]
  - 56 **Bos JL**, Fearon ER, Hamilton SR, Verlaan-de Vries M, van Boom JH, van der Eb AJ, Vogelstein B. Prevalence of ras gene mutations in human colorectal cancers. *Nature* 1987; **327**: 293-297 [PMID: 3587348 DOI: 10.1038/327293a0]
  - 57 **Gedde-Dahl T 3rd**, Fossum B, Eriksen JA, Thorsby E, Gaudernack G. T cell clones specific for p21 ras-derived peptides: characterization of their fine specificity and HLA restriction. *Eur J Immunol* 1993; **23**: 754-760 [PMID: 8449222 DOI: 10.1002/eji.1830230328]
  - 58 **Peace DJ**, Chen W, Nelson H, Cheever MA. T cell recognition of transforming proteins encoded by mutated ras proto-oncogenes. *J Immunol* 1991; **146**: 2059-2065 [PMID: 2005390]
  - 59 **Gjertsen MK**, Bakka A, Breivik J, Saeterdal I, Gedde-Dahl T 3rd, Stokke KT, Solheim BG, Egge TS, Søreide O, Thorsby E, Gaudernack G. Ex vivo ras peptide vaccination in patients with advanced pancreatic cancer: results of a phase I/II study. *Int J Cancer* 1996; **65**: 450-453 [PMID: 8621226 DOI: 10.1002/(SICI)1097-0215(19960208)65:4<450::AID-IJC10>3.0.CO;2-E]
  - 60 **Gjertsen MK**, Bakka A, Breivik J, Saeterdal I, Solheim BG, Søreide O, Thorsby E, Gaudernack G. Vaccination with mutant ras peptides and induction of T-cell responsiveness in pancreatic carcinoma patients carrying the corresponding RAS mutation. *Lancet* 1995; **346**: 1399-1400 [PMID: 7475823 DOI: 10.1016/S0140-6736(95)92408-6]
  - 61 **Shono Y**, Tanimura H, Iwahashi M, Tsunoda T, Tani M, Tanaka H, Matsuda K, Yamaue H. Specific T-cell immunity against Ki-ras peptides in patients with pancreatic and colorectal cancers. *Br J Cancer* 2003; **88**: 530-536 [PMID: 12592366 DOI: 10.1038/sj.bjc.6600697]
  - 62 **Khleif SN**, Abrams SI, Hamilton JM, Bergmann-Leitner E, Chen A, Bastian A, Bernstein S, Chung Y, Allegra CJ, Schlom J. A phase I vaccine trial with peptides reflecting ras oncogene mutations of solid tumors. *J Immunother* 1999; **22**: 155-165 [PMID: 10093040]
  - 63 **Wang QJ**, Yu Z, Griffith K, Hanada K, Restifo NP, Yang JC. Identification of T-cell Receptors Targeting KRAS-Mutated Human Tumors. *Cancer Immunol Res* 2016; **4**: 204-214 [PMID: 26701267 DOI: 10.1158/2326-6066.CIR-15-0188]
  - 64 **Toubaji A**, Ahtar M, Provenzano M, Herrin VE, Behrens R, Hamilton M, Bernstein S, Venzon D, Gause B, Marincola F, Khleif SN. Pilot study of mutant ras peptide-based vaccine as an adjuvant treatment in pancreatic and colorectal cancers. *Cancer Immunol Immunother* 2008; **57**: 1413-1420 [PMID: 18297281 DOI: 10.1007/s00262-008-0477-6]
  - 65 **Tran E**, Robbins PF, Lu YC, Prickett TD, Gartner JJ, Jia L, Pasetto A, Zheng Z, Ray S, Groh EM, Kriley IR, Rosenberg SA. T-Cell Transfer Therapy Targeting Mutant KRAS in Cancer. *N Engl J Med* 2016; **375**: 2255-2262 [PMID: 27959684 DOI: 10.1056/NEJMoa1609279]
  - 66 **Rahma OE**, Hamilton JM, Wojtowicz M, Dakheel O, Bernstein S, Liewehr DJ, Steinberg SM, Khleif SN. The immunological and clinical effects of mutated ras peptide vaccine in combination with IL-2, GM-CSF, or both in patients with solid tumors. *J Transl Med* 2014; **12**: 55 [PMID: 24565030 DOI: 10.1186/1479-5876-12-55]
  - 67 **Li Y**, Sahni N, Pancsa R, McGrail DJ, Xu J, Hua X, Coulombe-Huntington J, Ryan M, Tychon B, Sudhakar D, Hu L, Tyers M, Jiang X, Lin SY, Babu MM, Yi S. Revealing the Determinants of Widespread Alternative Splicing Perturbation in Cancer. *Cell Rep* 2017; **21**: 798-812 [PMID: 29045845 DOI: 10.1016/j.celrep.2017.09.071]
  - 68 **Gardina PJ**, Clark TA, Shimada B, Staples MK, Yang Q, Veitch J, Schweitzer A, Awad T, Sugnet C, Dee S, Davies C, Williams A, Turpaz Y. Alternative splicing and differential gene expression in colon cancer detected by a whole genome exon array. *BMC Genomics* 2006; **7**: 325 [PMID: 17192196 DOI: 10.1186/1471-2164-7-325]
  - 69 **Thorsen K**, Sørensen KD, Brems-Eskildsen AS, Modin C, Gaustadnes M, Hein AM, Kruhoffer M, Laurberg S, Borre M, Wang K, Brunak S, Krainer AR, Tørring N, Dyrskjøt L, Andersen CL, Orntoft TF. Alternative splicing in colon, bladder, and prostate cancer identified by exon array analysis. *Mol Cell Proteomics* 2008; **7**: 1214-1224 [PMID: 18353764 DOI: 10.1074/mcp.M700590-MCP200]
  - 70 Retained Introns Can Produce Tumor-Specific Neoepitopes in Melanoma. *Cancer Discov* 2018; **8**: 1206 [PMID: 30143520 DOI: 10.1158/2159-8290.CD-RW2018-144]
  - 71 **Ditzel HJ**, Strik MC, Larsen MK, Willis AC, Waseem A, Kejlting K, Jensenius JC. Cancer-associated cleavage of cytotokeratin 8/18 heterotypic complexes exposes a neoepitope in human adenocarcinomas. *J Biol Chem* 2002; **277**: 21712-21722 [PMID: 11923318 DOI: 10.1074/jbc.M202140200]
  - 72 **Koelzer VH**, Lugli A, Dawson H, Hädrich M, Berger MD, Borner M, Mallaev M, Galván JA, Amsler J, Schnüriger B, Zlobec I, Inderbitzin D. CD8/CD45RO T-cell infiltration in endoscopic biopsies of colorectal cancer predicts nodal metastasis and survival. *J Transl Med* 2014; **12**: 81 [PMID: 24679169 DOI: 10.1186/1479-5876-12-81]
  - 73 **Mogensen MB**, Rossing M, Østrup O, Larsen PN, Heiberg Engel PJ, Jørgensen LN, Hogdall EV, Eriksen J, Ibsen P, Jess P, Grauslund M, Nielsen HJ, Nielsen FC, Vainer B, Osterlind K. Genomic alterations accompanying tumour evolution in colorectal cancer: tracking the differences between primary tumours and synchronous liver metastases by whole-exome sequencing. *BMC Cancer* 2018; **18**: 752 [PMID: 30029640 DOI: 10.1186/s12885-018-4639-4]
  - 74 **Sylvester BE**, Vakiani E. Tumor evolution and intratumor heterogeneity in colorectal carcinoma: insights from comparative genomic profiling of primary tumors and matched metastases. *J Gastrointest Oncol* 2015; **6**: 668-675 [PMID: 26697200 DOI: 10.3978/j.issn.2078-6891.2015.083]
  - 75 **Bijker MS**, van den Eeden SJ, Franken KL, Melief CJ, van der Burg SH, Offringa R. Superior induction of anti-tumor CTL immunity by extended peptide vaccines involves prolonged, DC-focused antigen presentation. *Eur J Immunol* 2008; **38**: 1033-1042 [PMID: 18350546 DOI: 10.1002/eji.200737995]
  - 76 **Srinivasan M**, Domanico SZ, Kaumaya PT, Pierce SK. Peptides of 23 residues or greater are required to stimulate a high affinity class II-restricted T cell response. *Eur J Immunol* 1993; **23**: 1011-1016 [PMID: 8386663 DOI: 10.1002/eji.1830230504]
  - 77 **Eggermont LJ**, Paulis LE, Tel J, Figdor CG. Towards efficient cancer immunotherapy: advances in developing artificial antigen-presenting cells. *Trends Biotechnol* 2014; **32**: 456-465 [PMID: 24998519 DOI: 10.1016/j.tibtech.2014.06.007]
  - 78 **Awate S**, Babiuk LA, Mutwiri G. Mechanisms of action of adjuvants. *Front Immunol* 2013; **4**: 114 [PMID: 23720661 DOI: 10.3389/fimmu.2013.00114]
  - 79 **Parmiani G**, Castelli C, Pilla L, Santinami M, Colombo MP, Rivoltini L. Opposite immune functions of GM-CSF administered as vaccine adjuvant in cancer patients. *Ann Oncol* 2007; **18**: 226-232 [PMID: 17116643 DOI: 10.1093/annonc/mdl158]
  - 80 **Decker WK**, Safdar A. Cytokine adjuvants for vaccine therapy of neoplastic and infectious disease. *Cytokine Growth Factor Rev* 2011; **22**: 177-187 [PMID: 21862380 DOI: 10.1016/j.cytogfr.2011.07.001]
  - 81 **Dummer R**, Hauschild A, Henseler T, Burg G. Combined interferon-alpha and interleukin-2 as adjuvant treatment for melanoma.

- Lancet* 1998; **352**: 908-909 [PMID: 9743016 DOI: 10.1016/S0140-6736(05)60052-9]
- 82 **Rahma OE**, Gammoh E, Simon RM, Khleif SN. Is the “3+3” dose-escalation phase I clinical trial design suitable for therapeutic cancer vaccine development? A recommendation for alternative design. *Clin Cancer Res* 2014; **20**: 4758-4767 [PMID: 25037736 DOI: 10.1158/1078-0432.CCR-13-2671]
- 83 **Yoshida K**, Noguchi M, Mine T, Komatsu N, Yutani S, Ueno T, Yanagimoto H, Kawano K, Itoh K, Yamada A. Characteristics of severe adverse events after peptide vaccination for advanced cancer patients: Analysis of 500 cases. *Oncol Rep* 2011; **25**: 57-62 [PMID: 21109957 DOI: 10.3892/or\_00001041]
- 84 **Parkhurst MR**, Yang JC, Langan RC, Dudley ME, Nathan DA, Feldman SA, Davis JL, Morgan RA, Merino MJ, Sherry RM, Hughes MS, Kammula US, Phan GQ, Lim 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**: 620-626 [PMID: 21157437 DOI: 10.1038/mt.2010.272]
- 85 **Morgan RA**, Chinnasamy N, Abate-Daga D, Gros A, Robbins PF, Zheng Z, Dudley ME, Feldman SA, Yang JC, Sherry RM, Phan GQ, Hughes MS, Kammula US, Miller AD, Hessman CJ, Stewart AA, Restifo NP, Quezada MM, Alimchandani M, Rosenberg AZ, Nath A, Wang T, Bielekova B, Wuest SC, Akula N, McMahon FJ, Wilde S, Mosetter B, Schendel DJ, Laurencot CM, Rosenberg SA. Cancer regression and neurological toxicity following anti-MAGE-A3 TCR gene therapy. *J Immunother* 2013; **36**: 133-151 [PMID: 23377668 DOI: 10.1097/CJI.0b013e3182829903]
- 86 **Linette GP**, Stadtmauer EA, Maus MV, Rapoport AP, Levine BL, Emery L, Litzky L, Bagg A, Carreno BM, Cimino PJ, Binder-Scholl GK, Smethurst DP, Gerry AB, Pumphrey NJ, Bennett AD, Brewer JE, Dukes J, Harper J, Tayton-Martin HK, Jakobsen BK, Hassan NJ, Kalos M, June CH. Cardiovascular toxicity and titin cross-reactivity of affinity-enhanced T cells in myeloma and melanoma. *Blood* 2013; **122**: 863-871 [PMID: 23770775 DOI: 10.1182/blood-2013-03-490565]
- 87 **Whiteside TL**. The tumor microenvironment and its role in promoting tumor growth. *Oncogene* 2008; **27**: 5904-5912 [PMID: 18836471 DOI: 10.1038/ncr.2008.271]
- 88 **Zou W**. Immunosuppressive networks in the tumour environment and their therapeutic relevance. *Nat Rev Cancer* 2005; **5**: 263-274 [PMID: 15776005 DOI: 10.1038/nrc1586]
- 89 **Murdoch C**, Muthana M, Coffelt SB, Lewis CE. The role of myeloid cells in the promotion of tumour angiogenesis. *Nat Rev Cancer* 2008; **8**: 618-631 [PMID: 18633355 DOI: 10.1038/nrc2444]
- 90 **Ozcan M**, Janikovits J, von Knebel Doeberitz M, Kloor M. Complex pattern of immune evasion in MSI colorectal cancer. *Oncimmunology* 2018; **7**: e1445453 [PMID: 29900056 DOI: 10.1080/2162402X.2018.1445453]
- 91 **Atkins D**, Breuckmann A, Schmahl GE, Binner P, Ferrone S, Krummenauer F, Störkel S, Seliger B. MHC class I antigen processing pathway defects, ras mutations and disease stage in colorectal carcinoma. *Int J Cancer* 2004; **109**: 265-273 [PMID: 14750179 DOI: 10.1002/ijc.11681]
- 92 **Cabrera CM**, Jiménez P, Cabrera T, Esparza C, Ruiz-Cabello F, Garrido F. Total loss of MHC class I in colorectal tumors can be explained by two molecular pathways: beta2-microglobulin inactivation in MSI-positive tumors and LMP7/TAP2 downregulation in MSI-negative tumors. *Tissue Antigens* 2003; **61**: 211-219 [PMID: 12694570]
- 93 **Bicknell DC**, Kaklamanis L, Hampson R, Bodmer WF, Karran P. Selection for beta 2-microglobulin mutation in mismatch repair-defective colorectal carcinomas. *Curr Biol* 1996; **6**: 1695-1697 [PMID: 8994836]
- 94 **Bokhari A**, Jonchere V, Lagrange A, Bertrand R, Svrcek M, Marisa L, Buhard O, Greene M, Demidova A, Jia J, Adriaenssens E, Chassat T, Biard DS, Flejou JF, Lejeune F, Duval A, Collura A. Targeting nonsense-mediated mRNA decay in colorectal cancers with microsatellite instability. *Oncogenesis* 2018; **7**: 70 [PMID: 30228267 DOI: 10.1038/s41389-018-0079-x]
- 95 **Llosa NJ**, Cruise M, Tam A, Wicks EC, Hechenbleikner EM, Taube JM, Blosser RL, Fan H, Wang H, Lubber BS, Zhang M, Papadopoulos N, Kinzler KW, Vogelstein B, Sears CL, Anders RA, Pardoll DM, Housseau F. The vigorous immune microenvironment of microsatellite instable colon cancer is balanced by multiple counter-inhibitory checkpoints. *Cancer Discov* 2015; **5**: 43-51 [PMID: 25358689 DOI: 10.1158/2159-8290.CD-14-0863]
- 96 **Le DT**, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, Skora AD, Lubber BS, Azad NS, Laheru D, Biedrzycki B, Donehower RC, Zaheer A, Fisher GA, Crocenzi TS, Lee JJ, Duffy SM, Goldberg RM, de la Chapelle A, Koshiji M, Bhajee F, Huebner T, Hruban RH, Wood LD, Cuka N, Pardoll DM, Papadopoulos N, Kinzler KW, Zhou S, Cornish TC, Taube JM, Anders RA, Eshleman JR, Vogelstein B, Diaz LA Jr. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. *N Engl J Med* 2015; **372**: 2509-2520 [PMID: 26028255 DOI: 10.1056/NEJMoa1500596]
- 97 **Gupta R**, Sinha S, Paul RN. The impact of microsatellite stability status in colorectal cancer. *Curr Probl Cancer* 2018; **42**: 548-559 [PMID: 30119911 DOI: 10.1016/j.currprobcancer.2018.06.010]
- 98 **Rizvi NA**, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, Lee W, Yuan J, Wong P, Ho TS, Miller ML, Rekhtman N, Moreira AL, Ibrahim F, Bruggeman C, Gasmi B, Zappasodi R, Maeda Y, Sander C, Garon EB, Merghoub T, Wolchok JD, Schumacher TN, Chan TA. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science* 2015; **348**: 124-128 [PMID: 25765070 DOI: 10.1126/science.aaa1348]
- 99 **Snyder A**, Makarov V, Merghoub T, Yuan J, Zaretsky JM, Desrichard A, Walsh LA, Postow MA, Wong P, Ho TS, Hollmann TJ, Bruggeman C, Kannan K, Li Y, Elipenahli C, Liu C, Harbison CT, Wang L, Ribas A, Wolchok JD, Chan TA. Genetic basis for clinical response to CTLA-4 blockade in melanoma. *N Engl J Med* 2014; **371**: 2189-2199 [PMID: 25409260 DOI: 10.1056/NEJMoa1406498]
- 100 **Baker K**, Zlobec I, Tornillo L, Terracciano L, Jass JR, Lugli A. Differential significance of tumour infiltrating lymphocytes in sporadic mismatch repair deficient versus proficient colorectal cancers: a potential role for dysregulation of the transforming growth factor-beta pathway. *Eur J Cancer* 2007; **43**: 624-631 [PMID: 17223543 DOI: 10.1016/j.ejca.2006.11.012]
- 101 **Deschoolmeester V**, Baay M, Van Marck E, Weyler J, Vermeulen P, Lardon F, Vermorken JB. Tumor infiltrating lymphocytes: an intriguing player in the survival of colorectal cancer patients. *BMC Immunol* 2010; **11**: 19 [PMID: 20385003 DOI: 10.1186/1471-2172-11-19]
- 102 **Liu C**, Lu Z, Xie Y, Guo Q, Geng F, Sun B, Wu H, Yu B, Wu J, Zhang H, Yu X, Kong W. Soluble PD-1-based vaccine targeting MUC1 VNTR and survivin improves anti-tumor effect. *Immunol Lett* 2018; **200**: 33-42 [PMID: 29894719 DOI: 10.1016/j.imlet.2018.06.004]
- 103 **Vacchelli E**, Galluzzi L, Fridman WH, Galon J, Sautès-Fridman C, Tartour E, Kroemer G. Trial watch: Chemotherapy with immunogenic cell death inducers. *Oncimmunology* 2012; **1**: 179-188 [PMID: 22720239 DOI: 10.4161/onci.1.2.19026]
- 104 **Casares N**, Pequignot MO, Tesniere A, Ghiringhelli F, Roux S, Chaput N, Schmitt E, Hamai A, Hervas-Stubb S, Obeid M, Coutant F, Métivier D, Pichard E, Aucouturier P, Pierron G, Garrido C, Zitvogel L, Kroemer G. Caspase-dependent immunogenicity of doxorubicin-induced tumor cell death. *J Exp Med* 2005; **202**: 1691-1701 [PMID: 16365148 DOI: 10.1084/jem.20050915]
- 105 **Schiavoni G**, Sistigu A, Valentini M, Mattei F, Sestili P, Spadaro F, Sanchez M, Lorenzi S, D'Urso MT, Belardelli F, Gabriele L, Proietti E, Bracci L. Cyclophosphamide synergizes with type I interferons through systemic dendritic cell reactivation and induction of immunogenic tumor apoptosis. *Cancer Res* 2011; **71**: 768-778 [PMID: 21156650 DOI: 10.1158/0008-5473.CAN-10-2788]
- 106 **Tesniere A**, Schlemmer F, Boige V, Kepp O, Martins I, Ghiringhelli F, Aymeric L, Michaud M, Apetoh L, Barault L, Mendiboure J, Pignon JP, Jooste V, van Ender P, Ducreux M, Zitvogel L, Piard F, Kroemer G. Immunogenic death of colon cancer cells treated with oxaliplatin. *Oncogene* 2010; **29**: 482-491 [PMID: 19881547 DOI: 10.1038/onc.2009.356]
- 107 **Warner SG**, Haddad D, Au J, Carson JS, O'Leary MP, Lewis C, Monette S, Fong Y. Oncolytic herpes simplex virus kills stem-like tumor-initiating colon cancer cells. *Mol Ther Oncolytics* 2016; **3**:

- 16013 [PMID: 27347556 DOI: 10.1038/mto.2016.13]
- 108 **Yoo SY**, Bang SY, Jeong SN, Kang DH, Heo J. A cancer-favoring oncolytic vaccinia virus shows enhanced suppression of stem-cell like colon cancer. *Oncotarget* 2016; **7**: 16479-16489 [PMID: 26918725 DOI: 10.18632/oncotarget.7660]
- 109 **Andtbacka RH**, Ross MI, Agarwala SS, Taylor MH, Vetto JT, Neves RI, Daud A, Khong HT, Ungerleider RS, Tanaka M, Grossmann KF. Final results of a phase II multicenter trial of HF10, a replication-competent HSV-1 oncolytic virus, and ipilimumab combination treatment in patients with stage IIIB-IV unresectable or metastatic melanoma. *J Clin Oncol* 2017; **35**: 9510 [DOI: 10.1200/JCO.2017.35.15\_suppl.9510]
- 110 **Chesney J**, Puzanov I, Collichio F, Singh P, Milhem MM, Glaspy J, Hamid O, Ross M, Friedlander P, Garbe C, Logan TF, Hauschild A, Lebbé C, Chen L, Kim JJ, Gansert J, Andtbacka RHI, Kaufman HL. Randomized, Open-Label Phase II Study Evaluating the Efficacy and Safety of Talimogene Laherparepvec in Combination With Ipilimumab Versus Ipilimumab Alone in Patients With Advanced, Unresectable Melanoma. *J Clin Oncol* 2018; **36**: 1658-1667 [PMID: 28981385 DOI: 10.1200/JCO.2017.73.7379]
- 111 **Puzanov I**, Milhem MM, Minor D, Hamid O, Li A, Chen L, Chastain M, Gorski KS, Anderson A, Chou J, Kaufman HL, Andtbacka RH. Talimogene Laherparepvec in Combination With Ipilimumab in Previously Untreated, Unresectable Stage IIIB-IV Melanoma. *J Clin Oncol* 2016; **34**: 2619-2626 [PMID: 27298410 DOI: 10.1200/JCO.2016.67.1529]

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