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Review

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T-cell clonotypes in cancer

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Abstract

Cells of the immune system spontaneously recognize autologous tumor cells and T cells are believed to be the main effector cells for the immune surveillance of cancer. Recent advances in our understanding of basic and tumor immunology together with methodological developments implies that tumor specific T cells can now be studied functionally, phenotypically as well as molecularly. T cells recognize peptide antigens in the context of MHC molecules through the clonally distributed T-cell receptor (TCR), thus, the clonal distribution of the TCR offers the means to detect and track specific T cells based upon detection of the unique TCR. In this review, we present and discuss available data on TCR utilization of tumor specific T cells in murine models as well as spontaneous and treatment induced anti-tumor T-cell responses in humans.

Background

During the past decade our knowledge of the interplay between cancer cells and cells of the immune system has increased tremendously. In particular, advances in our understanding of T-cell recognition and priming, together with the processing and presentation of peptide antigens in the context of MHC molecules, has opened new avenues for characterization of antigens expressed by tumor cells – recognized by T cells. Since the first human tumor antigen recognized by T cells was described in melanoma more than a decade ago [1], an overwhelming number of human tumor antigens recognized by T cells has been characterized [2]. Over the past years, melanoma has consistently been the focus for extensive research in the field of tumor immunology – as clearly reflected by the data provided in present review. However, it has become clear that cancers in general elicit cellular immune responses [3]. Clearly, this has increased the potential clinical applicability of well characterized peptide antigens in the treatment of metastatic cancers, and thereby called further attention to the characterization of tumor associated peptide antigen. More importantly, however, new methods and technologies have provided scientists and clinicians with the means to dig deeper and faster. In this respect, the capacity to predict the peptides capable of binding to an HLA molecule [4], the introduction of methods to culture dendritic cells *in vitro* [5], highly sensitive methods for detection of specific T cells, e.g. ELISPOT [6], as well as the introduction of recombinant HLA molecules as tetrameric [7] or multimeric complexes [8], has played important roles. Following the characterization of peptide antigens recognized by autologous T cells on the surface of cancer cells, numerous small therapeutic vaccination trials against cancer have been performed aiming at the induction of clinically relevant anti-tumor CTL responses. Although some reports have demonstrated impressive clinical responses [9,10], in most cases vaccination has not yet convincingly demonstrated any impact on the

course of the disease [11]. In spite of the fact that clinical endpoints remain the most important success criteria for any given therapy, strategies that utilize biological modifiers in the treatment of disease may eventually benefit significantly from the increased insight that follows biological monitoring [12]. To this end, novel insight into the cells and molecules that govern the success or failure of any given therapy may lead the way to significant improvements of current therapeutic strategies. Consequently, characterization and tracking of the T-cell receptor (TCR) utilized by tumor specific T cells may reveal important information as to the biology of anti-tumor T-cell responses. In the present article we will review current knowledge on TCR repertoire usage and clonality of anti-tumor T-cell responses. The nomenclatur of Arden et al. is adopted throughout [13].

TCR repertoire analyses

Interest and early attempts to analyze tumor infiltrating lymphocytes (TIL) were initiated in part based on the fact that a crude infiltrate of T cells in melanoma lesions represents a positive prognostic factor [14]. While initial studies of TIL were solely dependent on immunhistochemisty, the introduction of the polymerase chain reaction (PCR) in a semi-quantitative setting for analyses of TCR variable regions set the stage for more comprehensive analyses of TIL [15,16]. Numerous repertoire analyses of various T-cell infiltrates have been conducted taking advantage of semi-quantitative analyses of TCRBV or AV usage [17,18]. The general findings provided by the use of semi-quantitative analyses of the variable regions expressed by TIL showed a skewed usage in comparison to peripheral blood lymphocytes (PBL) [17,19-21]. However, data from such analyses are – at best – indicative for an ongoing HLA restricted T-cell response, and the specificity of any potential responses is merely suggestive. Hence, although numerous studies included demonstration of clonality of TIL by cloning of TCR sequences followed by sequencing, or even by direct sequencing [17,19,22-25], the specificity and complexity of anti-tumor T cells were left unrevealed.

Methods for analyses of clonally expanded T cells

High resolution techniques for the detection of clonally expanded T cells are now available and established in numerous laboratories. In general, these methods are based on the RT-PCR amplification of T-cell receptor CDR3 regions using primers for the variable region in conjunction with a common primer for the constant part of the receptor, followed by detection of increased numbers of TCR transcripts of a specific T cell. Several methods are available for the swift detection and characterization of clonally expanded T cells [26-29]. However, the two most widespread methods are based on detection of increased numbers of TCR transcripts having 1) identical length (spectra typing or immunoscope) [18,30], or 2) distinct melting properties of the CDR3 region of the TCR (clonotype mapping) [31-33]. Both methods are established for human as well as murine TCRBV regions [30,34]. Similarly, both methods are well suited for detection as well as quantitation of specific clonally expanded T cells, and coupling to other techniques [35,36]. In addition, these methods provide the swift collection of PCR products for direct sequencing that in turn may readily form the basis for construction of clonotypic PCR primers for real time PCR analyses [36,37]. Summing up the general findings achieved by these high resolution techniques when employed for analyses of TIL, it has become clear that the T-cell infiltrate in tumor lesions comprise high numbers of clonally expanded T cells [22,31], and unpublished observations.

Since these early attempts to study the involvement of specific T-cell clonotypes in the immune response against cancer cells, several important developments have promoted the direct *ex vivo* assessments of tumor specific T cells. First, the characterization of a high number of HLA restricted peptides and the use of such peptides in vaccinations against cancer imply that treatment induced specific T cells are readily analyzed over the course of treatment [38]. Second, the introduction of recombinant HLA molecules as tetra – or multimeric complexes coupled with specific peptides, and the possibility to use these constructs for direct analyses and isolations of peptide specific T cells enabled the clonotypic characterization of such populations [36,39]. Finally, the means to employ the ELISPOT methodology for functional analyses of T cells made it possible to correlate functional aspects with the results of analyses addressing the TCR repertoire usage and clonality obtained by spectra typing or clonotype mapping [36,40].

T-cell receptor utilization of tumor specific T cells in murine models

Although murine tumor models have been utilized in cancer immunotherapy for several decades in pre-clinical experiments, very limited data is available with regards to TCR usage and clonality of tumor specific T cells; neither spontaneously nor during therapy. Similarly, the number of class I restricted tumor associated peptides characterized in murine tumors is devastating small compared to human cancers [2,41-47]. Consequently, only a limited number of murine studies have been conducted in which TCRs of known specificity are scrutinized. The DBA/2 murine mastocytoma model P815 has been analyzed for the TCR clonotypes involved in anti-tumor CTL responses against specific antigens, taking advantage of the spectra typing. Analyses of the infiltrate in this model revealed a highly variable clonality among different mice. However, a striking finding in this setting was the involvement of

two recurrent TCR rearrangements in the *in vivo* response against P815 – emerging in almost all animals [48]. Direct sequencing demonstrated that each of these "clonotypes" were not absolutely monoclonal, but nevertheless in several instances different CDR3 sequences coded for identical protein sequences, suggesting a strong selection for the TCR protein in question. Moreover, these two recurrent TCR rearrangements – called "public" – were present during tumor progression as well as tumor rejection. One of the TCR clonotypes recognized an epitope of the cancer/testis antigen P1A [42]. Clearly, the recurrent selection of identical TCRs in any given response suggests the involvement of high affinity receptors [49], in turn indicating that normal non-mutated self antigens may give rise to T-cell responses that involve high affinity TCRs, at least when studied in murine models. The stepwise progression of human cancers implies that mechanisms of peripheral tolerance may be at play – in transplanted murine tumors such mechanisms are not likely to play an equally significant role. Interestingly, the antigen recognized by the second public TCR rearrangement was subsequently characterized and shown to be a peptide which results from a point mutation in the methionine sulfoxide reductase (MsrA) [41].

Numerous studies of immunotherapy have been conducted in the B16 melanoma model. Again, however, only few studies have been conducted to analyze for T-cell clonality against B16; treated as well as non-treated. TCR clonotype mapping was used to analyze the clonality of the T-cell infiltrate of B16 tumor treated with antibody targeted IL-2. Compared to the P815 there was no evidence for the involvement of public responses, but clonality was only analyzed for the BV regions 5, 8, and 12 [50]. A more recent study included the full panel of TCRBV regions in clonotype analyses for studies of spontaneous T-cell responses against B16 melanoma. In this study, more than 600 clonotypic transcripts were compared, and none of these clonotypes could recurrently be identified in more than a single animal. Moreover, in this line of experiments three tumors were induced in each animal, and only a very limited number of clonotypes were detected in more than one tumor in each animal. Hence, even when analyzed in the same animal the response to each tumor was largely unique [32].

In the same model, we recently analyzed T-cell responses against B16 tumors when IL-2 is targeted to the tumor microenvironment by means of specific antibody-IL-2 fusion proteins. Interestingly, the therapeutic effect of the fusion protein was not restricted to tumors expressing the targeted antigen, but extends to antigen negative variants of the tumor in the same animal. Analysis of the T-cell infiltrate by quantitative reverse transcription-PCR revealed the presence of highly expressed TCR BV regions in both tumor variants. TCR clonotype mapping revealed that the high expressions of these regions were caused by clonal expansions and, notably, that these specific clonotypic TCR transcripts were identical in both tumors. Thus, compared to the data from the analyses of non-treated B16 lesions this suggests that T-cell clones activated locally by targeted IL-2 therapy gain the capacity to re-circulate and mediate eradication of distant tumor sites not subjected to *in situ* cytokine therapy [34]. However, the specificity and consequently direct evidence for the involvement of the detected clonotypes in an ongoing anti-tumor T cell was not revealed.

Using the same targeting approach against B16 melanomas, targeting of lymphotoxin α to the tumor site was demonstrated to lead to the induction of an efficient immune response, involving increased numbers of T-cell clones among TIL over the course of treatment [51]. Data from the analysis of the dynamics of the individual T-cell clonotypes during treatment demonstrated the induction of new clones during treatment but also the presence of persistent of T-cell clones. Taking advantage of ELISPOT analyses, the infiltrate in the tumors and draining lymph nodes were demonstrated to comprise CTL specific for the $K^b/TRP2_{180–188}$ complex. Importantly, TRP2 specific CTL responses were present in treated as well as un-treated mice. Although specific reactivity was ten times higher in treated animals this demonstrates that these responses develop spontaneously [51].

A more comprehensive characterization of the T-cell clonotypes in the response against tumor-associated antigens in murine models awaits the molecular characterization of the relevant peptides.

T-cell receptor utilization of tumor specific T cells in humans: spontaneous responses

As mentioned, several studies have been performed in which the *in vivo* clonality of TIL – in particular in melanoma – have been analyzed. Over the past few years it has become well recognized that T-cell responses develop spontaneously not only in melanoma but in other cancers as well; that most if not all cancers elicit cellular responses [3]. Nevertheless, it appears that some antigens are spontaneously immunogenic, e.g., survivin, her-2/neu, MUC-1, the melanocyte differentiation antigens gp100 and Mart-1 [3], and ML-IAP [52], whereas other antigens – like most members of the Mage family – apparently do not elicit a measurable natural T-cell response [3].

More than a decade ago, Sensi and colleges established tumor specific CTL clones from HLA-A2 positive melanoma patients [53]. By analyzing the lytic activity against various melanoma target cells they demonstrated

that TCRAV2/BV2 and TCRAV2/BV14 CTL clones were restricted by the HLA-A2 molecule. Importantly, these CTLs were shown to react not only against melanoma cells but also against normal HLA-A2 positive melanocytes. As suggested by the data given below this indicates that these CTL specifically recognized the Mart-1/HLA-A2 peptide, characterized independently by two research groups the following year (1994) [54,55].

Clearly, antigens that elicit a spontaneous CTL response offers the means to scrutinize these responses and reveal further insight into the biology of natural immune responses against cancer. Following the demonstration that *in vitro* established Mart-1/HLA-A2 specific CTL frequently express TCRBV14 [56], Salvi and colleges used semi-quantitative PCR to analyze for expression of TCRBV regions in tumor biopsies from HLA-A2 positive and negative melanoma patients [21]. Based on the assumption that Mart-1 is immunodominant in the T-cell response against melanoma and at the same time not knowing whether other factors (e.g., other peptides also preferentially recognized by TCRBV14 T cells) may have influenced the predominance of TCRBV14 T cells, they demonstrated a direct correlation between numbers of TCRBV14 utilizing T cells and Mart-1 protein expression in melanoma lesions in HLA-A0201 positive patients. In spite of the fact that the clonality of the involved T cells was not studied, it pointed to a scenario in which Mart-1 specific T cells – preferentially expressing TCRBV14 – took part in the *in situ* anti-tumor response against melanoma cells.

The long list of characterized human peptide antigens expressed and presented on the surface of cancer cells, offers the means to scrutinize the *in situ* presence of peptide specific CTL. However, in some cases CTL clonotypes reactive against autologous tumor cells – but nevertheless of unknown peptide specificity – have been characterized. In this respect, Mackensen et al. detected a TCRBV16 CTL clone *in situ* in a case of regressive melanoma. The restriction element of the the antigen was revealed to be HLA-B14, but the protein and the peptide was not characterized [57]. Likewise, Pisarra el al., demonstrated tumor specificity and cytotoxic capacity of an *in situ* expanded TCRBV14 CTL clonotype in a HLA-A2 melanoma patient. As given above, Mart-1 specific CTL predominantly express BV14, however, neither the restriction element nor the peptide was characterized [58].

More recent developments have offered the means to combine specificiy of the T cell with analyses of clonality. Valmori and colleges conducted a tetramer guided analysis of the TCRBV region usage of Mart-1 specific T cells [59], followed by a study from the same group focusing on TCRBV chain clonality of these Mart-1 specific T cells [60]. The general findings of these studies demonstrated that the anti-Mart-1 T-cell response was highly diverse although T cells expressing the BV14 region were predominant; thus, confirming earlier reports from Salvi et al. [21]. The involvement of numerous TCRs recognizing the same HLA/peptide comples demonstrates the *in vivo* availability – and selection – of a high number of different TCRs recognizing one HLA/peptide complex. Thus, these data confirm the *in vivo* relevance of data from Romero et al. demonstrating that *in vitro* established HLA-A1/Mage-1 specific CTL may possess profound differences in their fine specificity [61]. This also suggests lack of strong selection for specific TCRs in the response; conversely to the results obtained by analyzing the response against the MsrA and the P1A antigens in the murine P815 model, no recurrent TCR sequences were identified among the twelve patients analyzed [60]. Notably, the presence of such selections, i.e. public TCRs, have only been described in human T-cell responses directed against viral peptides [62]. The background for the apparent discrepancy of viral and anti-tumor responses remains unknown. One possibility is that high affinity TCRs against self-antigens like Mart-1 are removed during selection in the thymus, or even by tumor-induced deletion [63], thereby eliminating potentially dominant TCR clonotypes. Still, rather few TCRs reactive against specific HLA/peptide complexes have been extensively studied. Consequently, it is possible that any conserved TCR usage could be more related to the biochemical properties of the peptide than to the origin of the peptide.

The occurrence of melanoma associated hypopigmentation is believed to be caused by T-cell responses specific for melanocyte differentiation antigens, e.g., gp100 or Mart-1. Indeed, by clonotype mapping we could demonstrate the existence of clonally expanded T cells with identical BV regions accumulating in areas of destruction of both normal, i.e. melanocytes, and neoplastic cells, i.e. tumor cells [64]. As these clones supposedly are reactive against antigens expressed on normal as well as neoplastic cells, this observation demonstrates the quite unlimited potential of the positively selected T-cell repertoire for immunotherapy. To this end, vililigo patients have been demonstrated to possess high frequencies of Mart-1 specific CTL [65]. In a recent study, Mantovani et al. scrutinized the Mart-1 CTL response in a vitiligo patient, and demonstrated that a single TCRAV region constituted the alpha chain in all Mart-1 specific CTL clones, and also that these CTL persisted for more than 3 years in the patient [66].

Interestingly, T cells participating in the immune response against cancer are not restricted to the tumor site and the sentinel lymph node. In this regard, we previously demonstrated the recurrent indentification of a T-cell clone in

melanoma lesions presenting in the patient with two years interval. Moreover, the T-cell clone were present in the sentinal lymph node of the first as well as the second lesion [67]. Thus, the sentinel lymph node harbor T cells that recognise the tumor, but the clones present in the excised tissue were nevertheless maintained for almost 2 years in other compartments.

T-cell receptor utilization of tumor specific T cells in humans: treatment induced responses

Administration of cytokines has been used for the treatment of malignant disease for many years. In this regard, interleukin-2 is used for the treatment of malignant melanoma as well as renal cell carcinoma, and systemic administration of high dose IL-2 induce complete regression in a small but significant proportion of the patients [68]. In spite of the widespread use of cytokines in clinical oncology, not much is known with regards to the precise immunological mechanisms associated with the *in vivo* efficacy of IL-2 administration. A recent study suggested that high dose IL-2 leads to activation of monocytes and NK cells at the tumor site, in turn leading to the recruitment of T cells [69]. However, very few studies have focused on analyses of T cells over the course of cytokine administration. We recently used the ELISPOT assay to analyze for specific T-cell responses in melanoma patients receiving low dose s.c. IL-2, in combination with local electro-chemotherapy [36].

Surprisingly, specific reactivity in PBL as measured against peptides derived from Mart-1, gp100, TRP-2, and survivin decreased during IL-2 administration but re-occurred between each IL-2 cycle. Intriguingly, analyses of the clonotype composition of Mart-1 specific T cells by clonotype mapping demonstrated that new clonotypes occurred during treatment and that TRP-2 specific T cells detected in the blood could subsequently be detected at the tumor site. Together this indicates that IL-2 adds to the capacity of T cells to home to the tumor site, and – importantly – questions the information gained alone by analyses of blood samples. In a recent study, Willhauck et al. studied the TCRBV repertoire of regressing melanoma lesions after administration of IL-2 and INF-α [70]. However, as in the above mentioned study direct evidence that establishes the link between the achieved data and the treatment is lacking [20,71]. Nevertheless, in some respects, analyses of immune reactivity during IL-2 represents an ideal model that could be used to gain novel insight into clinically relevant anti-tumor immune responses; the targets recognized, the phenotype and clonotypic composition of tumor specific CTL, ect., that may lead to improvements of more sophisticated immune therapeutic strategies, e.g., therapeutic vaccinations against cancer.

Sensi and colleges studied clonal expansions of T cells in melanoma metastases after vaccination with hapten-modified autologous tumor cells [72]. Data from studies of pre- and post vaccine tumor lesions demonstrated a more intense inflammation, and also that expression levels of specific TCRBV regions were markedly changed in post-vaccine lesions. From one patient, several lesions were analyzed over a two-year period and distinct clonotypes were recurrently detected. Furthermore, TIL lines predominantly expressing TCRBV14 were capable of lyzing autologous melanoma cells. Together this suggests a treatment induced response, substantiated by data from a subsequent study of *in situ* T-cell clonality in melanoma patients treated with DNP-modified autologous tumor cells [73]. Examination of TCR repertoire and clonality in post-vaccine metastases demonstrated a highly diverse picture though specific clonotypes comprised up to more than half of all T cells expressing the BV14 region. Strikingly, specific clonotypes could recurrently be detected in all (four) lesions in one of the patients.

The above mentioned data were recently further substantiated by more comprehensive data on treatment induced TCR clonality in response to vaccination with hapten-modified autologous melanoma cells [74]. Thus, in 9 of 10 patients, analyses of post-vaccine biopsies showed dominant expansions in several BV families, one of which was BV14. Again, identical TCRs were detectable in biopsies obtained at different times or sites during treatment. Clearly, these data suggest that the detected T-cell expansions are indeed induced by the treatment. Although the final prove for this notion is still lacking, it indicates that vaccination based on autologous tumor cells induces a highly diverse T-cell response, at least as judged by TCR usage of responding T cells. Whether this indicates a correspondingly diverse specificity of the response remain unrevealed.

For studies of specific T-cell responses, e.g., clonality of the response, peptide based vaccinations offer an ideal setting. Such vaccinations have been ongoing for more than a decade. The first peptide based vaccination trial used subcutaneous administration of crude peptide [75], while more recent peptide based vaccinations against cancer have taken advantage of various adjuvants in combination with peptide(s). However, data from studies of TCR utilization are only available for very few peptide specificities. In this regard, Valmori et al. analyzed the induced T-cell response in blood of melanoma patients vaccinated with Mart-$1_{26–35}$ peptide administered i.m. with SB-AS2 as adjuvant [76]. Characterization of the BV clonotype composition of T cells stained with the Mart-1 tetramer over the course of treatment, demonstrated a predominance of T cells expressing BV1, 3, 5, 14, and 17. These cells were expanded *in vitro* for three to four weeks,

offering the opportunity to conduct functional analyses. The downside of *in vitro* culture is obvious; not all CTLs – for some populations probably only a minority – will be supported by *in vitro* growth conditions [77,78]. However, this shortcoming was taken into account in the study of Valmori et al., by comparing the BV repertoire and CDR3 spectra before and after *in vitro* culture. Thus, although the BV1 T cells were lost in culture, most *in vivo* clonotypes were preserved. Subsequent sequencing offered the means to investigate whether Mart-1 T cells detected in peripheral blood could recurrently be detected at the tumor site, and indeed this was the case. Also, the tracking of specific clonotypes provided the means to gain insight into the dynamics of the induced CTL response, demonstrating a highly asynchronous expansion of responding T cells. Interestingly, the functional avidity increased during vaccination, however, the mechanism(s) were left unrevealed. Clearly, it would be of interest to evaluate the clonotypes comprised in the spontaneous Mart-1 response and compare these clonotypes to those induced by treatment to reveal whether the increase in functional avidity was merely due to induction of T cells not taking part in the spontaneous response. To this end, Terheyden et al., conducted a longitudinal study of Mart-1 specific T cells over the course of melanoma progression in a patient vaccinated with dendritic cells pulsed with peptides [79]. The tracking of Mart-1 clonotypes from onset of treatment demonstrated the persistence of TCRBV2 and BV14 clonotypes [80]. Moreover, the percentage of TCRBV14 cells capable of producing INF-γ before treatment was 9%, but increased to 52% during treatment, and declined to 11% upon severe disease progression. Clearly, neither the study of Valmori et al., nor the study by Terheyden and colleges studied the complete Mart-1 specific CTL population. Still, it points to a scenario in which vaccination may not only induce the expansion of CTL not involved in the spontaneous response, but possibly also alter the functionality of pre-existing specific CTL.

Vaccinations using peptides derived from cancer/testis antigens, in particular peptides derived from Mage proteins, have been ongoing for more than a decade, and the T-cell response intensively studied [81]. In two recent reports the clonality of vaccine induced Mage-specific T cells were analyzed [82,83]. Godelaine and colleges scrutinized the T-cell repertoire in four melanoma patients vaccinated with DCs loaded with Mage-3/HLA-A1 peptide [83]. Taking advantage of tetrameric complexes, specific T cells were cloned, expanded *in vitro*, and the TCRB sequence determined. In three of the four patients examined, a specific response was induced and TCR clonality analyzed. In each patient Mage-3/HLA-A1 specific T-cell populations were characterized by expression of several distinct TCR chains, but also that a single TCR comprised from above 30 to app. 50 % to of the responding cells. Thus, although several discrete T cells were activated during vaccination, some clones were clearly more abundant. Coulie et al. analyzed a single patient vaccinated with the same HLA-A1 restricted peptide, however, administered s.c. and intradermally as crude peptide [82]. There was a vigorous increase in the frequency of specific CTL, however, this T-cell response was monoclonal, as judged after *in vitro* stimulation. The patient experienced regression of several metastatic sites, but also simultaneous progression of others. Tracking by clonotypic PCR demonstrated that this specific clone was present even at progressive tumor sites and studies of the expression of antigen and HLA by the tumor cells, demonstrated that (some of...) the prerequisites for T-cell recognition were fulfilled. The different results concerning clonality of the two studies mentioned above raises the question whether this is a reflection of DC based vaccination *versus* the use of crude peptide.

Considering the very limited number of patients analyzed it remains to be elucidated whether these marked differences in clonality are reflections of the two vaccination protocols – or merely differences among the patients. However, it could be speculated that the DC based approach are more prone to provide sufficient help for the expansion of CTLs of relative low affinity – and that the crude peptide approach only activates a narrow repertoire of T cells carrying high affinity receptors. Our own previous results for DC based vaccination sustain this hypothesis regarding DCs. In fact, *in vitro* matured and antigen-pulsed DCs inoculated in the skin are able to induce a peptide specific oligoclonal T-cell response in the dermal compartment [84]. Nevertheless, these studies emphasize the strength of combining the use of recombinant HLA molecules with clonotype analyses of responding T cells.

Berger and colleges studied the TCR repertoire in a melanoma patient vaccinated with HLA-A2, and HLA-A24 restricted peptides of several tumor antigens, i.e., peptides from Mart-1, tyrosinase, and Mage-3 [85]. Mart-1 specific T cells – as determined by staining with multimeric peptide/HLA complexes – were present in the lesions prior to vaccination, but the numbers increased substantially after vaccination. Moreover, Mage-3/HLA-A2 specific T cells were not detectable prior to vaccination, but became apparent after vaccination. Interestingly, analyses of the clonal composition of the T-cell infiltrate in a cutaneous and a visceral lesion demonstrated the presence of identical TCR clonotypes in these two lesions. Clearly, this indicates that distinct homing marker profiles may develop during the response – most likely independent of the priming event. It should be noted that the T-cell clone present in the metastases could be detected in the circulation as well; however, no quantification of these cells were performed, thus, no correlation between circulating and

in situ T-cell responses can be drawn from this observation.

Another immunotherapeutic approach in cancer is the adoptive transfer of (potentially) tumor specific T cells. Early attempts pursueing this approach took advantage of *in vitro* expansion of autologous TIL or PBL and the subsequent transfer of cells to the patient. However, despite some success in preclinial models [86], the response rates observed in the human setting were not encouraging [87]. However, the adoptive transfer of *in vitro* expanded TIL in combination with high-dose IL-2, administered subsequent to non-myeloablative chemotherapy, was recently demonstrated to induce objective clinical responses in 6 out of 13 melanoma patients [88]. Importantly, all cultures were analyzed for the capacity to recognize autologous or HLA-matched tumor cell targets prior to administration. Data from TCR repertoire analyses of the adminstered TIL cultures demonstrated at skewed BV region usage in five of six patients. Moreover, in two of the patients specific T-cell clonotypes were monitored and demonstrated to expand *in vivo*. Thus, in these two patients Mart-1/HLA-A2 T-cell clones comprised more than 60% of the CD8 population for more than 4 months. Clearly, such high frequencies are way above CTL frequencies obtained by any other means of immunotherapy against cancer, and indeed several of the patients experienced autoimmunity (vitiligo/uveitis). Strikingly, a similar treatment strategy was previously persued by the same group, however, by administration of *in vitro* expanded T-cell clones [89]; none of the 15 patients included responded clinically. Although larger trials are required to make firm conclusions, it could point to a significant role of CD4 cells in the administered cultures – in turn underlining the importance of establishing methods for monitoring and tracking of tumor specific CD4 T-cell clonotypes [90].

Analyses of TCRBV versus *TCRAV: Does it matter?*

Most studies addressing the TCR repertoire usage have been conducted with an emphasis on BV regions. Considering PCR based methods this is actually not optimal: there is a high degree of homology between the different BV regions, and several BV families comprise numerous members, implying that specific PCR amplification of all family members without cross reactivity to other BV families, is not entirely trivial. Conversely, TCRAV families comprise relatively few members and homology between the families is low compared to TCRB families [13]. Early data on virus specific T cells and TCRBV usage, demonstrated a preferential usage of specific BV regions; these data may have influenced the field with regards to whether AV or BV chains should be studied [62,91]. Irrespective of the reason, most researchers have conducted clonality and repertoire analyses of the TCRB chain. Recently, a dominant role of the TCRAV chain in the recognition of HLA/peptide complexes was demonstrated [92-94]. Analyses of V region usage and CDR3 region conservation in T cells specific for viral as well as tumor associated peptide/HLA-A2 revealed a recurrent use of AV regions, a more heterogeneous usage of BV regions, and highly diverse junctional sequences [92]. Likewise, Trautman and colleges found a frequent usage of TCRBV14 by Mart-1 specific T cells, but a much more prominent usage of TCRAV2.1. Strikingly, Dietrich et al. similarly analyzed Mart-1_{27-35}/HLA-A2 specific T cells and found a strong selection for TCRAV2.1 also in cord blood, out ruling that the narrow repertoire was developed by affinity focusing [93].

These data suggests a prominent role for the TCRA chain for determining the specificity of the receptor. In some respects this seems self explanatory due to the fact that the TCRB chain re-arrange prior to the TCRA chain. This implies that any TCRB may eventually be associated with multiple TCRA chains. Consequently, if the TCRB were the important determinant of specificity, T cells carrying identical TCRB and different TCRA would have a high risk for identical specificity. In any case, final prove for any general TCRA – or B bias and their roles as determinants of specificity awaits further TCR/peptide/MHC structures to be revealed [95]. Interestingly, it was recently demonstrated that a "public" TCR is highly predominant in CTL responses against the dominant EBV peptide restricted by HLA-B8, and germline encoded parts of the TCR was demonstrated to be involved in recognition [96,97]. This touches on whether such TCRs – heavily influenced by germline sequences – are evolutionarily selected, and therefore such responses could possibly be quite different than responses against e.g., self antigens associated with cancer.

Concluding remarks

Data from molecular analyses of T-cell clonotypes in various clinical infiltrates have over the past years contributed to our understanding of cellular immune responses. However, whereas previous data were merely descriptive, present days technical and methodological advances offer the possibility to by combine phenotype and functional data with analyses of TCR repertoire and clonality. "Molecular fingerprinting" of T cells with known specificity offers the means to reveal a more sophisticated and instructive picture of ongoing T-cell responses by covering spatial and temporal dynamics. Certainly, such data will significantly increase our understanding of the cells and molecules that govern anti-cancer T-cell responses thereby leading the way to improve immune therapeutic strategies to combat cancer.

List of abbreviations

Beta variable (BV), denaturing gradient gel electrophoresis (DGGE), T-cell receptor (TCR), tumor infiltrating lymphocyte (TIL), interleukin-2 (IL-2), cytotoxic T lymphocyte (CTL), dinitrophenyl (DNP), complementarity-determining region 3 (CDR3).

Competing interests

None declared

Authors' contributions

All authors contributed with information and data. All authors read and approved the manuscript.

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References

1. van der Bruggen P, Traversari C, Chomez P, Lurquin C, De Plaen E, Van den Eynde B, Knuth A, Boon T: **A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma.** *Science* 1991, **254:**1643-1647.
2. Renkvist N, Castelli C, Robbins PF, Parmiani G: **A listing of human tumor antigens recognized by T cells.** *Cancer Immunol Immunother* 2001, **50:**3-15.
3. Nagorsen D, Scheibenbogen C, Marincola FM, Letsch A, Keilholz U: **Natural T cell immunity against cancer.** *Clin Cancer Res* 2003, **9:**4296-4303.
4. Rammensee HG, Falk K, Roetzschke O: **MHC molecules as peptide receptors.** *Curr Biol* 1995, **5:**35-44.
5. Schuler G, Schuler-Thurner B, Steinman RM: **The use of dendritic cells in cancer immunotherapy.** *Curr Opin Immunol* 2003, **15:**138-147.
6. Schmittel A, Keilholz U, Thiel E, Scheibenbogen C: **Quantification of tumor-specific T lymphocytes with the ELISPOT assay.** *J Immunother* 2000, **23:**289-295.
7. Altman JD, Moss PA, Goulder PJR, Barouch DH, McHeyzer Williams MG, Bell JI, McMichael AJ, Davis MM: **Phenotypic analysis of antigen-specific T lymphocytes.** *Science* 1996, **274:**94-96.
8. Andersen MH, Ostergaard Pedersen L, Capeller B, Bröcker EB, Becker JC, thor Straten P: **Spontaneous cytoxic T-cell responses against survivin-derived MHC class I restricted T-cell epitopes in situ as well as ex vivo in cancer patients.** *Cancer Res* 2001, **61:**5964-5968.
9. Nestle FO, Alijagic S, Gilliet M, Sun Y, Grabbe S, Dummer R, Burg G, Schadendorf D: **Vaccination of melanoma patients with peptide- or tumor lysate-pulsed dendritic cells.** *Nat Med* 1998, **4:**328-332.
10. Rosenberg SA, Yang JC, Schwartzentruber DJ, Hwu P, Marincola FM, Topalian SL, Restifo NP, Dudley ME, Schwarz SL, Spiess PJ, Wunderlich JR, Parkhurst MR, Kawakami Y, Seipp CA, Einhorn JH, White DE: **Immunologic and therapeutic evaluation of a synthetic peptide vaccine for the treatment of patients with metastatic melanoma.** *Nat Med* 1998, **4:**321-327.
11. Parmiani G, Pilla L, Castelli C, Rivoltini L: **Vaccination of patients with solid tumours.** *Ann Oncol* 2003, **14:**817-824.
12. Keilholz U, Weber J, Finke JH, Gabrilovich DI, Kast WM, Disis ML, Kirkwood JM, Scheibenbogen C, Schlom J, Maino VC, Lyerly HK, Lee PP, Storkus W, Marincola FM, Worobec A, Atkins MB: **Immunologic monitoring of cancer vaccine therapy: results of a workshop sponsored by the Society for Biological Therapy.** *J Immunother* 2002, **25:**97-138.
13. Arden B, Clark SP, Kabelitz D, Mak TW: **Human T-cell receptor variable gene segment families.** *Immunogenetics* 1995, **42:**455-500.
14. Clark WH: **Tumour progression and the nature of cancer.** *Br J Cancer* 1991, **64:**631-644.
15. Nitta T, Oksenberg JR, Rao NA, Steinman RM: **Predominant expression of T cell receptor V alpha 7 in tumor- infiltrating lymphocytes of uveal melanoma.** *Science* 1990, **249:**672-674.
16. Oksenberg JR, Stuart S, Begovich AB, Bell RB, Erlich HA, Steinman RM, Bernard CC: **Limited heterogeneity of rearranged T-cell receptor V alpha transcripts in brains of multiple sclerosis patients [published erratum appears in Nature 1991 Sep 5;353(6339):94].** *Nature* 1990, **345:**344-346.
17. Sensi M, Parmiani G: **Analysis of TCR usage in human tumors:a new tool for assessing tumor-specific immune responses.** *Immunol Today* 1995, **16:**588-595.
18. Pannetier C, Even J, Kourilsky P: **T-cell repertoire diversity and clonal expansions in normal and clinical samples.** *Immunol Today* 1995, **16:**176-181.
19. thor Straten P, Schøller J, Hou Jensen K, Zeuthen J: **Preferential usage of T-cell receptor alpha beta variable regions among tumor-infiltrating lymphocytes in primary human malignant melanomas.** *Int J Cancer* 1994, **56:**78-86.
20. thor Straten P, Becker JC, Seremet T, Bröcker EB, Zeuthen J: **Clonal T-cell responses in tumor infiltrating lymphocytes from both regressive and progressive regions of primary human melanoma.** *J Clin Invest* 1996, **98:**279-284.
21. Salvi S, Segalla F, Rao S, Arienti F, Sartori M, Bratina G, Caronni E, Anichini A, Clemente C, Parmiani G, Sensi M: **Overexpression of the T-cell receptor beta-chain variable region TCRBV14 in HLA-A2-matched primary human melanomas.** *Cancer Res* 1995, **55:**3374-3379.
22. Puisieux I, Even J, Pannetier C, Jotereau F, Favrot M, Kourilsky P: **Oligoclonality of tumor-infiltrating lymphocytes from human melanomas.** *J Immunol* 1994, **153:**2807-2818.
23. Ferradini L, Roman Roman S, Azocar J, Avril MF, Viel S, Triebel F, Hercend T: **Analysis of T-cell receptor alpha/beta variability in lymphocytes infiltrating a melanoma metastasis.** *Cancer Res* 1992, **52:**4649-4654.
24. Ferradini L, Mackensen A, Genevee C, Bosq J, Duvillard P, Avril MF, Hercend T: **Analysis of T cell receptor variability in tumor-infiltrating lymphocytes from a human regressive melanoma. Evidence for in situ T cell clonal expansion.** *J Clin Invest* 1993, **91:**1183-1190.
25. Mackensen A, Ferradini L, Carcelain G, Triebel F, Faure F, Viel S, Hercend T: **Evidence for in situ amplification of cytotoxic T-lymphocytes with antitumor activity in a human regressive melanoma.** *Cancer Res* 1993, **53:**3569-3573.
26. Hishii M, Andrews D, Boyle LA, Wong JT, Pandolfi F, van den Elsen PJ, Kurnick JT: **In vivo accumulation of the same anti-melanoma T cell clone in two different metastatic sites.** *Proc Natl Acad Sci USA* 1997, **94:**1378-1383.
27. Wack A, Montagna D, Dellabona P, Casorati G: **An improved PCR-heteroduplex method permits high-sensitivity detection of clonal expansions in complex T cell populations.** *J Immunol Methods* 1996, **196:**181-192.
28. Bousso P, Kourilsky P: **A clonal view of alphabeta T cell responses.** *Semin Immunol* 1999, **11:**423-431.
29. thor Straten P, Barfoed A, Seremet T, Säterdal I, Zeuthen J, Guldberg P: **Detection and Characterization of alpha/beta-T-cell Clonality by Denaturing Gradient Gel Electrophoresis (DGGE).** *Biotechniques* 1998, **25:**244-250.
30. Pannetier C, Cochet M, Darche S, Casrouge A, Zöller M, Kourilsky P: **The sizes of the CDR3 hypervariable regions of the murine T-cell receptor beta chains vary as a function of the recombined germ-line segments.** *Proc Natl Acad Sci USA* 1993, **90:**4319-4323.
31. thor Straten P, Guldberg P, Grønbæk K, Zeuthen J, Becker JC: **In Situ T-Cell Responses against Melanoma Comprise High Numbers of Locally Expanded T-Cell Clonotypes.** *J Immunol* 1999, **163:**443-447.
32. Moerch U, Schrama D, Guldberg P, Seremet T, Zeuthen J, Becker JC, thor Straten P: **Comparative delineantion of T cell clonotypes in coexisting syngeneic B16 melanoma.** *Cancer Immunol Immunother* 2000, **49:**426-432.
33. thor Straten P, Becker JC, Zeuthen J, Guldberg P: **T-cell receptor clonotype mapping using denaturing gradient gel electrophoresis (DGGE): Analyses of clonal T-cell responses in**

melanoma. *Melanoma Methods and Protocols* Edited by: NicholoffB. Humana Press. Inc.; 2001.

34. thor Straten P, Guldberg P, Hald Andersen M, Moerch U, Seremet T, Siedel C, Zeuthen J, Reisfeld RA, Becker JC: **In situ cytokine therapy: Redistribution of clonally expanded therapeutic T-cell clones.** *Eur J Immunol* 2001, **31:**250-258.
35. Pilch H, Hohn H, Freitag K, Neukirch C, Necker A, Haddad P, Tanner B, Knapstein PG, Maeurer MJ: **Improved assessment of T-cell receptor (TCR) VB repertoire in clinical specimens: combination of TCR-CDR3 spectratyping with flow cytometry-based TCR VB frequency analysis.** *Clin Diagn Lab Immunol* 2002, **9:**257-266.
36. Andersen MH, Gehl J, Reker S, Ostergaard Pedersen L, Becker JC, Geertsen PF, thor Straten P: **Dynamic changes of specific T cell responses to melanoma correlate with IL-2 administration.** *Semin Cancer Biol* 2003, **13:**449-459.
37. Lim A, Baron V, Ferradini L, Bonneville M, Kourilsky P, Pannetier C: **Combination of MHC-peptide multimer-based T cell sorting with the Immunoscope permits sensitive ex vivo quantitation and follow-up of human CD8+ T cell immune responses.** *J Immunol Methods* 2002, **261:**177-194.
38. Parmiani G, Castelli C, Dalerba P, Mortarini R, Rivoltini L, Marincola FM, Anichini A: **Cancer immunotherapy with peptide-based vaccines: what have we achieved? Where are we going?** *J Natl Cancer Inst* 2002, **94:**805-818.
39. Schrama D, Andersen MH, Terheyden P, Schrøder L, Ostergaard Pedersen L, thor Straten P, Becker JC: **Oligoclonal TCR usage of melanocyte differentiation antigen-reactive T cells.** *Cancer Res* 2001, **61:**493-496.
40. Dietrich PY, Walker PR, Quiquerez AL, Perrin G, Dutoit V, Lienard D, Guillaume P, Cerottini JC, Romero P, Valmori D: **Melanoma patients respond to a cytotoxic T lymphocyte-defined self-peptide with diverse and nonoverlapping T-cell receptor repertoires.** *Cancer Res* 2001, **61:**2047-2054.
41. Bilsborough J, Van Pel A, Uyttenhove C, Boon T, Van den Eynde BJ: **Identification of a second major tumor-specific antigen recognized by CTLs on mouse mastocytoma P815.** *J Immunol* 1999, **162:**3534-3540.
42. Lethe B, van den Eynde B., Van Pel A, Corradin G, Boon T: **Mouse tumor rejection antigens P815A and P815B: two epitopes carried by a single peptide.** *Eur J Immunol* 1992, **22:**2283-2288.
43. Overwijk WW, Tsung A, Irvine KR, Parkhurst MR, Goletz TJ, Tsung K, Carroll MW, Liu C, Moss B, Rosenberg SA, Restifo NP: **gp100/pmel 17 is a murine tumor rejection antigen: induction of "self"- reactive, tumoricidal T cells using high-affinity, altered peptide ligand.** *J Exp Med* 1998, **188:**277-286.
44. Siegel S, Wagner A, Schmitz N, Zeis M: **Induction of antitumour immunity using survivin peptide-pulsed dendritic cells in a murine lymphoma model.** *Br J Haematol* 2003, **122:**911-914.
45. Bloom MB, Perry-Lalley D, Robbins PF, Li Y, El-Gamil M, Rosenberg SA, Yang JC: **Identification of tyrosinase-related protein 2 as a tumor rejection antigen for the B16 melanoma.** *J Exp Med* 1997, **185:**453-459.
46. Vierboom MP, Nijman HW, Offringa R, van der Voort EI, van Hall T, van den Broek L., Fleuren GJ, Kenemans P, Kast WM, Melief CJ: **Tumor eradication by wild-type p53-specific cytotoxic T lymphocytes.** *J Exp Med* 1997, **186:**695-704.
47. Mumberg D, Wick M, Schreiber H: **Unique tumor antigens redefined as mutant tumor-specific antigens.** *Semin Immunol* 1996, **8:**289-293.
48. Levraud JP, Pannetier C, Langlade-Demoyen P, Brichard V, Kourilsky P: **Recurrent T cell receptor rearrangenments in the cytotoxic T lymphocyte response In Vivo against P815 murine tumor.** *J Exp Med* 1996, **183:**439-449.
49. Cibotti R, Cabaniols JP, Pannetier C, Delarbre C, Vergnon I, Kanellopoulos JM, Kourilsky P: **Public and private V beta T cell receptor repertoires against hen egg white lysozyme (HEL) in non-transgenic versus HEL transgenic mice.** *J Exp Med* 1994, **180:**861-872.
50. thor Straten P, Guldberg P, Seremet T, Reisfeld RA, Zeuthen J, Becker JC: **Activation of pre-existing T-cell clones by targeted interleukin 2 therapy.** *Proc Natl Acad Sci USA* 1998, **95:**8785-8790.
51. Schrama D, thor Straten P, Fischer WH, Merkel A, Bröcker EB, Reisfeld RA, Becker JC: **Targeting lymphotoxin alpha to the tumor microenvironment elicits an efficient immune response by induction of peripheral lymphoid tissue.** *Immunity* 2001, **14:**111-121.
52. Andersen MH, Reker S, Becker JC, thor Straten P: **The melanoma inhibitor of apoptosis protein: a target for spontaneous cytotoxic T cell responses.** *J Invest Dermatol* 2004, **122:**392-399.
53. Sensi M, Salvi S, Castelli C, Maccalli C, Mazzocchi A, Mortarini R, Nicolini G, Herlyn M, Parmiani G, Anichini A: **T cell receptor (TCR) structure of autologous melanoma-reactive cytotoxic T lymphocyte (CTL) clones: tumor-infiltrating lymphocytes overexpress in vivo the TCR beta chain sequence used by an HLA-A2-restricted and melanocyte-lineage-specific CTL clone.** *J Exp Med* 1993, **178:**1231-1246.
54. Kawakami Y, Eliyahu S, Delgado CH, Robbins PF, Rivoltini L, Topalian SL, Miki T, Rosenberg SA: **Cloning of the gene coding for a shared human melanoma antigen recognized by autologous T cells infiltrating into tumor.** *Proc Natl Acad Sci USA* 1994, **91:**3515-3519.
55. Coulie PG, Brichard V, Van Pel A, Wölfel T, Schneider J, Traversari C, Mattei S, De Plaen E, Lurquin C, Szikora JP, et al: **A new gene coding for a differentiation antigen recognized by autologous cytolytic T lymphocytes on HLA-A2 melanomas.** *J Exp Med* 1994, **180:**35-42.
56. Sensi M, Traversari C, Radrizzani M, Salvi S, Maccalli C, Mortarini R, Rivoltini L, Farina C, Nicolini G, Wölfel T, et al: **Cytotoxic T-lymphocyte clones from different patients display limited T-cell-receptor variable-region gene usage in HLA-A2- restricted recognition of the melanoma antigen Melan-A/MART-1.** *Proc Natl Acad Sci USA* 1995, **92:**5674-5678.
57. Mackensen A, Carcelain G, Viel S, Raynal MC, Michalaki H, Triebel F, Bosq J, Hercend T: **Direct evidence to support the immunosurveillance concept in a human regressive melanoma.** *J Clin Invest* 1994, **93:**1397-1402.
58. Pisarra P, Mortarini R, Salvi S, Anichini A, Parmiani G, Sensi M: **High frequency of T cell clonal expansions in primary human melanoma. Involvement of a dominant clonotype in autologous tumor recognition.** *Cancer Immunol Immunother* 1999, **48:**39-46.
59. Valmori D, Dutoit V, Lienard D, Lejeune F, Speiser D, Rimoldi D, Cerundolo V, Dietrich PY, Cerottini JC, Romero P: **Tetramer-guided analysis of TCR beta-chain usage reveals a large repertoire of melan-A-specific CD8+ T cells in melanoma patients.** *J Immunol* 2000, **165:**533-538.
60. Dietrich PY, Walker PR, Quiquerez AL, Perrin G, Dutoit V, Lienard D, Guillaume P, Cerottini JC, Romero P, Valmori D: **Melanoma patients respond to a cytotoxic T lymphocyte-defined self-peptide with diverse and nonoverlapping T-cell receptor repertoires.** *Cancer Res* 2001, **61:**2047-2054.
61. Romero P, Pannetier C, Herman J, Jongeneel CV, Cerottini JC, Coulie PG: **Multiple specificities in the repertoire of a melanoma patients cytolytic T lymphocytes directed against tumor antigen MAGE-1.A1.** *J Exp Med* 1995, **182:**1019-1028.
62. Lehner PJ, Wang EC, Moss PA, Williams S, Platt K, Friedman SM, Bell JI, Borysiewicz LK: **Human HLA-A0201-restricted cytotoxic T lymphocyte recognition of influenza A is dominated by T cells bearing the V beta 17 gene segment.** *J Exp Med* 1995, **181:**79-91.
63. Molldrem JJ, Lee PP, Kant S, Wieder E, Jiang W, Lu S, Wang C, Davis MM: **Chronic myelogenous leukemia shapes host immunity by selective deletion of high-avidity leukemia-specific T cells.** *J Clin Invest* 2003, **111:**639-647.
64. Becker JC, Guldberg P, Zeuthen J, Bröcker EB, thor Straten P: **Accumulation of identical T cells in melanoma and vitiligo-like leukoderma.** *J Invest Dermatol* 1999, **113:**1033-1038.
65. Ogg GS, Rod Dunbar P., Romero P, Chen JL, Cerundolo V: **High frequency of skin-homing melanocyte-specific cytotoxic T lymphocytes in autoimmune vitiligo.** *J Exp Med* 1998, **188:**1203-1208.
66. Mantovani S, Garbelli S, Palermo B, Campanelli R, Brazzelli V, Borroni G, Martinetti M, Benvenuto F, Merlini G, della Cuna GR, Rivoltini L, Giachino C: **Molecular and functional bases of self-antigen recognition in long-term persistent melanocyte-specific CD8+ T cells in one vitiligo patient.** *J Invest Dermatol* 2003, **121:**308-314.
67. Schrama D, Eggert AA, Brocker EB, Pedersen LO, thor Straten P, Becker JC: **Immunological consequences of the sentinel lymph-node biopsy--lessons from a melanoma patient.** *Lancet Oncol* 2003, **4:**446-447.

68. Atkins MB: **Interleukin-2: clinical applications.** *Semin Oncol* 2002, **29:**12-17.
69. Panelli MC, Wang E, Phan G, Puhlmann M, Miller L, Ohnmacht GA, Klein HG, Marincola FM: **Gene-expression profiling of the response of peripheral blood mononuclear cells and melanoma metastases to systemic IL-2 administration.** *Genome Biol* 2002, **3:**RESEARCH0035.
70. Willhauck M, Scheibenbogen C, Pawlita M, Mohler T, Thiel E, Keilholz U: **Restricted T-cell receptor repertoire in melanoma metastases regressing after cytokine therapy.** *Cancer Res* 2003, **63:**3483-3485.
71. Schrama D, Fuchs E, Brocker EB, thor Straten P, Becker JC: **Identical T-cell receptor transcripts in multiple melanoma metastases.** *Cancer Res* 2002, **62:**5664-5667.
72. Sensi M, Farina C, Maccalli C, Lupetti R, Nicolini G, Anichini A, Parmiani G, Berd D: **Clonal expansion of T lymphocytes in human melanoma metastases after treatment with a hapten-modified autologous tumor vaccine.** *J Clin Invest* 1997, **99:**710-717.
73. Sensi M, Farina C, Maccalli C, Anichini A, Berd D, Parmiani G: **Intralesional selection of T cell clonotypes in the immune response to melanoma antigens occurring during vaccination.** *J Immunother* 1998, **21:**198-204.
74. Manne J, Mastrangelo MJ, Sato T, Berd D: **TCR rearrangement in lymphocytes infiltrating melanoma metastases after administration of autologous dinitrophenyl-modified vaccine.** *J Immunol* 2002, **169:**3407-3412.
75. Marchand M, Weynants P, Rankin E, Arienti F, Belli F, Parmiani G, Cascelli N, Bourlond A, Vanwijck R, Humblet Y, Canon JL, Laurent C, Naeyaert JM, Plangne R, Deraemaeker R, Knuth A, Jäger E, Brasseur F, Herman J, Coulie PG, Boon T: **Tumor regression responses in melanoma patients treated with a peptide encoded by gene MAGE-3.** *Int J Cancer* 1995, **63:**883-885.
76. Valmori D, Dutoit V, Schnuriger V, Quiquerez AL, Pittet MJ, Guillaume P, Rubio-Godoy V, Walker PR, Rimoldi D, Lienard D, Cerottini JC, Romero P, Dietrich PY: **Vaccination with a Melan-A peptide selects an oligoclonal T cell population with increased functional avidity and tumor reactivity.** *J Immunol* 2002, **168:**4231-4240.
77. thor Straten P, Kirkin AF, Siim E, Dahlstrom K, Drzewiecki KT, Seremet T, Zeuthen J, Becker JC, Guldberg P: **Tumor infiltrating lymphocytes (TIL) in melanoma comprise high numbers of T-cell clonotypes that are lost during in vitro culture.** *Clin Immunol* 2000, **96:**94-99.
78. Faure F, Even J, Kourilsky P: **Tumor-specific immune response: current in vitro analyses may not reflect the in vivo immune status.** *Crit Rev Immunol* 1998, **18:**77-86.
79. Pass HA, Schwarz SL, Wunderlich JR, Rosenberg SA: **Immunization of patients with melanoma peptide vaccines: immunologic assessment using the ELISPOT assay.** *Cancer J Sci Am* 1998, **4:**316-323.
80. Terheyden P, Schrama D, Pedersen LO, Andersen MH, Kampgen E, thor Straten P, Becker JC: **Longitudinal analysis of MART-1/HLA-A2-reactive T cells over the course of melanoma progression.** *Scand J Immunol* 2003, **58:**566-571.
81. Coulie PG, Karanikas V, Lurquin C, Colau D, Connerotte T, Hanagiri T, Van Pel A, Lucas S, Godelaine D, Lonchay C, Marchand M, van Baren N, Boon T: **Cytolytic T-cell responses of cancer patients vaccinated with a MAGE antigen.** *Immunol Rev* 2002, **188:33-42.:**33-42.
82. Coulie PG, Karanikas V, Colau D, Lurquin C, Landry C, Marchand M, Dorval T, Brichard V, Boon T: **A monoclonal cytolytic T-lymphocyte response observed in a melanoma patient vaccinated with a tumor-specific antigenic peptide encoded by gene MAGE-3.** *Proc Natl Acad Sci USA* 2001, **98:**10290-10295.
83. Godelaine D, Carrasco J, Lucas S, Karanikas V, Schuler-Thurner B, Coulie PG, Schuler G, Boon T, Van Pel A: **Polyclonal CTL responses observed in melanoma patients vaccinated with dendritic cells pulsed with a MAGE-3.A1 peptide.** *J Immunol* 2003, **171:**4893-4897.
84. Schrama D, Ostergaard Pedersen L, Keikavoussi P, Andersen MH, thor Straten P, Brocker EB, Kampgen E, Becker JC: **Aggregation of antigen-specific T cells at the inoculation site of mature dendritic cells.** *J Invest Dermatol* 2002, **119:**1443-1448.
85. Berger TG, Haendle I, Schrama D, Lüftl M, Bauer N, Ostergaard Pedersen L, Schuler-Thurner B, Hohenberger W, thor Straten P, Schuler G, Becker JC: **Circulation and homing of melanoma-reactive T cell clones to both cutaneaus and visceral metastases.** *Int J Cancer* 2004, **In Press.:**.
86. Rosenberg SA, Spiess PJ, Lafreniere R: **A new approach to the adoptive immunotherapy of cancer with tumor- infiltrating lymphocytes.** *Science* 1986, **233:**1318-1321.
87. Rosenberg SA, Yannelli JR, Yang JC, Topalian SL, Schwartzentruber DJ, Weber JS, Parkinson DR, Seipp CA, Einhorn JH, White DE: **Treatment of patients with metastatic melanoma with autologous tumor- infiltrating lymphocytes and interleukin 2.** *J Natl Cancer Inst* 1994, **86:**1159-1166.
88. Dudley ME, Wunderlich JR, Robbins PF, Yang JC, Hwu P, Schwartzentruber DJ, Topalian SL, Sherry R, Restifo NP, Hubicki AM, Robinson MR, Raffeld M, Duray P, Seipp CA, Rogers-Freezer L, Morton KE, Mavroukakis SA, White DE, Rosenberg SA: **Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes.** *Science* 2002, **298:**850-854.
89. Dudley ME, Wunderlich JR, Yang JC, Hwu P, Schwartzentruber DJ, Topalian SL, Sherry RM, Marincola FM, Leitman SF, Seipp CA, Rogers-Freezer L, Morton KE, Nahvi A, Mavroukakis SA, White DE, Rosenberg SA: **A phase I study of nonmyeloablative chemotherapy and adoptive transfer of autologous tumor antigen-specific T lymphocytes in patients with metastatic melanoma.** *J Immunother* 2002, **25:**243-251.
90. Kwok WW: **Challenges in staining T cells using HLA class II tetramers.** *Clin Immunol* 2003, **106:**23-28.
91. Moss PA, Moots RJ, Rosenberg WM, Rowland-Jones SJ, Bodmer HC, McMichael AJ, Bell JI: **Extensive conservation of alpha and beta chains of the human T-cell antigen receptor recognizing HLA-A2 and influenza A matrix peptide.** *Proc Natl Acad Sci U S A* 1991, **88:**8987-8990.
92. Trautmann L, Labarriere N, Jotereau F, Karanikas V, Gervois N, Connerotte T, Coulie PG, Bonneville M: **Dominant TCR V alpha usage by virus and tumor-reactive T cells with wide affinity ranges for their specific antigens.** *Eur J Immunol* 2002, **32:**3181-3190.
93. Dietrich PY, Le Gal FA, Dutoit V, Pittet MJ, Trautman L, Zippelius A, Cognet I, Widmer V, Walker PR, Michielin O, Guillaume P, Connerotte T, Jotereau F, Coulie PG, Romero P, Cerottini JC, Bonneville M, Valmori D: **Prevalent role of TCR alpha-chain in the selection of the preimmune repertoire specific for a human tumor-associated self-antigen.** *J Immunol* 2003, **170:**5103-5109.
94. Mantovani S, Palermo B, Garbelli S, Campanelli R, Robustelli della Cuna G., Gennari R, Benvenuto F, Lantelme E, Giachino C: **Dominant TCR-alpha requirements for a self antigen recognition in humans.** *J Immunol* 2002, **169:**6253-6260.
95. Bankovich AJ, Garcia KC: **Not just any T cell receptor will do.** *Immunity* 2003, **18:**7-11.
96. Argaet VP, Schmidt CW, Burrows SR, Silins SL, Kurilla MG, Doolan DL, Suhrbier A, Moss DJ, Kieff E, Suclley TB: **Dominant selection of an invariant T cell antigen receptor in response to persistent infection by Epstein-Barr virus.** *J Exp Med* 1994, **180:**2335-2340.
97. Kjer-Nielsen L, Clements CS, Purcell AW, Brooks AG, Whisstock JC, Burrows SR, McCluskey J, Rossjohn J: **A structural basis for the selection of dominant alphabeta T cell receptors in antiviral immunity.** *Immunity* 2003, **18:**53-64.