

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800

Open access books available

122,000

International authors and editors

135M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Immunotherapy of Renal Cell Carcinoma – From Antigen Identification to Patient Treatment

Heiko Schuster¹, Mathias Walzer^{1,2} and Stefan Stevanović¹

¹Department of Immunology, Institute for Cell Biology, University of Tübingen

²Applied Bioinformatics Group, Center for Bioinformatics, University of Tübingen
Germany

1. Introduction

Renal cell carcinoma (RCC) is the 3rd most common urologic cancer leading to an estimated 271 000 new cancer cases worldwide each year (Ferlay *et al.* 2010). RCC is generally associated with poor prognosis because of late diagnosis and bad responsiveness to classical radio- and chemotherapy. For a long period of time immunotherapy with IL-2 or Interferon alpha (IFN α) have been the only therapeutic options to treat advanced stage RCCs. In recent years targeted therapy with tyrosine kinase inhibitors (TKIs; Sunitinib, Sorafenib) or mTor inhibitors (Everolimus, Temsirolimus) has replaced cytokine based immunotherapy as a first line treatment in the management of metastatic RCCs (mRCC). IFN α is nevertheless still playing an important role in the treatment of mRCC not as a monotherapy, but rather in combination with the antibody Bevacizumab which is directed against proangiogenic vascular endothelial growth factor (VEGF).

Despite these recent improvements resistance to this new class of drugs frequently occurs and curative treatment of RCC is still only possible by surgical resection of the tumor mass at an early non-metastatic stage (Rini & Atkins 2009). A lot of effort has therefore been put into the development of a targeted immunotherapeutic approach that aims at the *in vivo* induction or reinforcement of an anti tumor immune response.

2. Cancer immunotherapy in the course of history

The underlying idea that the immune system is able to recognise and kill transformed cells is actually not new (Parish 2003). As early as the 19th century William Coley and others noticed that cancer patients suffering from a bacterial infection sometimes experienced tumor regression. William Coley was also the first to translate this observation into clinical practice by treating cancer patients with a bacterial preparation of *Streptococcus pyogenes* known as Coley's toxin (Coley 1893). In 1909 Paul Ehrlich evaluated the role of the immune system in tumor control and suggested that without the immune system cancers would occur in much higher frequency (Ehrlich 1909). Unfortunately it took more than half a century before Ehrlich's idea regained attention. First experimental evidence during the 1950s could show that syngenic animals could be immunized against transplantable tumors. In the early 1960s Lewis Thomas argued that long-lived organisms must have developed mechanisms that resemble homograft rejection in order to counter neoplastic diseases

(Lawrence 1959). Based on Thomas' views Frank M. Burnet formulated in 1967 his revolutionary „Immunosurveillance Theory“ in which he stated that immune cells were constantly surveying host tissues for the presence of transformed cells that could be recognized by neo-antigens acquired during the transformation process (Burnet 1967). However Burnet's idea remained controversial simply because many people could not believe that the immune system could properly differentiate between healthy tissue and transformed cancer cells. Experiments with athymic nude mice also argued against Burnet's idea (Stutman 1979). Despite their supposed lack of mature T cells (which was later shown to be not completely true (Maleckar & Sherman 1987)) these mice had no higher incidence in tumor development as originally predicted by Ehrlich. Since the late 80s, more and more evidence accumulated that revived the immunosurveillance theory. The discovery of tumor associated antigens (TAA) in mice and humans as well as the observation that truly immunodeficient knockout mice (*e.g.* RAG2^{-/-}, STAT1^{-/-}) really showed a higher incidence in cancer development finally proved Burnet's idea.

3. Immunotherapeutical studies in RCC

RCC, along with melanoma, are considered to be the most immunogenic tumor in humans. This is based on the occurrence of spontaneous regressions even in metastatic disease (Lokich 1997), the high amount of lymphocytic infiltrates found in tumor tissue (Van den Hove *et al.* 1997) and the comparably good response to nonspecific immunotherapy with cytokines like IL-2 or IFN α , at least in some part of the patients (10-20%) (Negrier *et al.* 1998). First clinical trials using a specific immunotherapy for RCC were therefore conducted during the mid - 90s already. Table 1 provides a comprehensive summary of published results regarding anti-tumor vaccination approaches from that time until today. Many different strategies involving lysate from autologous tumor tissue, allogenic tumor cell lines, whole tumor cell RNA as well as defined antigens (*e.g.* peptides derived from TAAs CAIX or MUC1) have been used. In most cases these antigens were applied in combination with an adjuvant (*e.g.* GM-CSF, BCG or incomplete Freund's adjuvant) or after loading onto dendritic cells (DCs) usually from the same patient. Dendritic cells are professional antigen presenting cells that have the capacity to take up tumor derived products spontaneously (in some trials also enforced by electrofusion with tumor cells) and to present antigen derived peptides *via* HLA class I and II molecules to cytotoxic- and also T helper cells. In most trials dendritic cells were generated from autologous peripheral blood derived monocytes or CD34⁺ bone marrow cells that can both develop *ex vivo* into immature dendritic cells (iDCs). More recent studies almost uniformly apply a cytokine maturation step to generate mature dendritic cells (mDCs) as iDCs have been shown to possess tolerogenic rather than immune stimulatory functionality (Figdor *et al.* 2004). Keyhole limpet hemocyanin has been also applied in many trials as an immunostimulant because it was previously shown to improve dendritic cell based vaccinations by inducing a potent CD4⁺ T helper cell response at least in mouse models (Shimizu *et al.* 2001). Other approaches to enhance the immunogenicity of the vaccine have been tried by virally transducing autologous tumor cells and allogenic cell lines with cytokines (GM-CSF, IL2) or T-cell costimulatory molecule CD80 (B7.1).

Vaccination with all these different approaches has proven to be well tolerated and side effects have been rare and were usually limited to allergic reactions at the site of injection as well as induration or erythema. In trials in which additional cytokine treatment (IL-2, IFN α) was employed, further side effects like fatigue, fever, vomiting and hypotension could be observed.

In some cases systemic application of high dose cytokines can however also lead to serious and life-threatening side effects. These symptoms have been known for a long time to also occur under cytokine monotherapy and are therefore not directly associated with the vaccine.

Inactivated tumor cells and gene modified tumor vaccines:					
Author	Setting	Antigen	Adjuvant	N*	Results
(Galligioni <i>et al.</i> 1996)	Adjuvant after nephrectomy	Autol. Irradiated tumor cells	BCG	60 (60)	5-year DFS 63% vs. control 72%; 5-year OS 69% vs. control 78%
(Simons & Mikhak 1998)	mRCC	Autol. Irradiated tumor cells transduced with GM-CSF	None	16	1 PR
(Schwaab <i>et al.</i> 2000)	metastatic RCC	Autol. Irradiated tumor cells	BCG IFNg+ IFNa	14	3 MR, 5 SD, 1 PD
Dillman(Dillman <i>et al.</i> 2004)	8 primary RCC, 17 mRCC	Autol. Irradiated tumor cells	BCG, GM-CSF, IFNa, IFNg, IL2, CP	25	median PFS 2.4 months, median OS 10,2 months
(Jocham <i>et al.</i> 2004)	Adjuvant after nephrectomy	Autol. tumor lysate vaccine (Reniale)	None	177 (202)	5 year PFS 77.4% vs control 67.8%
(Dudek <i>et al.</i> 2008)	stage IV RCC	Autol. large multivalent immunogen vaccine	none, CP, IL-2 + CP	31	median PFS 12.2, 1 PR, 12 SD
(May <i>et al.</i> 2009)	Adjuvant after nephrectomy	Autol. tumor lysate vaccine (Reniale)	None	495 (495)	5-year OS 80.6% vs. control 79,2%, 10-year OS 79,2% vs. control 62,1%
(Antonia <i>et al.</i> 2002)	mRCC	Autol. Irradiated tumor cells transduced with B7-1	IL-2	15	2 PR, 2SD
(Tani <i>et al.</i> 2004)	stage IV RCC	Autol. Irradiated tumor cells transduced with GM-CSF	none, low dose IL-2	4	1 SD, 1MR
(Pizza <i>et al.</i> 2004)	mRCC	Autol. fixed tumor cells + allog. ACHN cell line transduced with IL-2	None	30 (131)	1 CR, 4 PR, 9 SD
(Moiseyenko <i>et al.</i> 2005)	mRCC after cytoreductive surgery	Autol. Irradiated tumor cells transfected with tag7/PGRP-S	None	4	1 SD
(Fishman <i>et al.</i> 2008)	stage IV RCC	Autol. Irradiated tumor cells transduced with B7-1	IL-2	39	1 CR, 24 SD
(Buchner <i>et al.</i> 2010)	mRCC	Allog.RCC-26 tumor cell line transduced with CD80 and IL 2	None	15	median PFS 5.3 months, median OS 15.6 months

Peptide based vaccines					
Authors	Setting	Antigen	Adjuvant	N*	Results
(Uemura <i>et al.</i> 2006)	mRCC, HLA-A*24 pos.	CA9 derived peptides (p219-227, p288-296, p323-331)	IFA	23	3 PR, 6SD, median OS 21 months
(Iiyama <i>et al.</i> 2007)	mRCC, HLA-A*24 pos.	WT1 anchor modified peptide	IFA	3	2 SD
(Suekane <i>et al.</i> 2007)	mRCC, HLA-A*24 pos or HLA-A*2 pos.	Personalized cocktail of 4 peptides based on presence of Anti Peptide IgG or CTLs	IFA	10	6 SD
(Wood <i>et al.</i> 2008)	Adjuvant after nephrectomy	Autol. tumor derived heat-shock protein-peptide complex (Vitespen)	None	367 (361)	No difference in RFS
(Jonasch <i>et al.</i> 2008)	mRCC	Autol. tumor derived heat-shock protein-peptide complex (Vitespen)	None, IL2	60	2 CR, 2PR, 7SD
(Reinhardt <i>et al.</i> 2010)	mRCC, HLA-A*02 pos.	IMA901, 9 HLA-A*02 + 1 panDR restricted peptides	GMCSF, single dose CP before vaccination	64	OS after 18 months 63% and 80% with CP pretreatment

Dendritic cell based vaccines					
Authors	Setting	DC + Antigen	Adjuvant/ Additional Treatment	N*	Results
(Oosterwijk-Wakka <i>et al.</i> 2002)	mRCC	Autol. iDCs loaded with autol. tumor cell lysate	IL-2;/ DC pulsed with KLH,none	12	8 SD
(Marten <i>et al.</i> 2002)	mRCC	Autol. mDCs loaded with autol. tumor cell lysate	none;/ DC pulsed with KLH, none	15	1 PR, 7 SD
(Holtl <i>et al.</i> 2002)	mRCC	Autol. mDCs loaded with autol. tumor cell lysate or allog. A498 cell line lysate	none / DC pulsed with KLH	35	2 CR, 1 PR, 7 SD
(Azuma <i>et al.</i> 2002)	mRCC	Autol. iDCs loaded with autol. tumor cell lysate	none / DC pulsed with KLH	3	1 SD
(Marten <i>et al.</i> 2003)	mRCC	Allog. mDCs fused with autol. tumor cells	None	12	4 SD
(Marten <i>et al.</i> 2006)	mRCC, HLA matched	Autol. mDCs loaded with a telomerase derived peptide	none; DC pulsed with KLH	10	1 MR, 1 SD
(Gitlitz <i>et al.</i> 2003)	mRCC	Autol. iDCs loaded with autol. tumor cell lysate	None	12	1 PR, 3 SD
(Barbuto <i>et al.</i> 2004)	mRCC	Allog. mDCs fused with autol. tumor derived cells	None	19	3 OR, 14 SD
(Avigan <i>et al.</i> 2007)	stage IV RCC	Allog. mDCs fused with autol. tumor derived cells	None	20	2 PR, 8 SD
(Avigan <i>et al.</i> 2004)	mRCC	Allog. iDCs fused with autol. tumor derived cells	DC pulsed with KLH	13	5 SD
(Pandha <i>et al.</i> 2004)	mRCC	Autol. iDCs loaded with allog. lysate of tumor cell line JM-RCC	KLH	5	2 SD
(Arroyo <i>et al.</i> 2004)	mRCC	Autol. mDCs loaded with autol. tumor cell lysate	DC pulsed with KLH	5	3 SD
(Dannull <i>et al.</i> 2005)	mRCC	Autol. mDCs transfected with whole tumor RNA	Pretreatment with ONTAK (Treg depletion)	11	not evaluated
(Holtl <i>et al.</i> 2005)	mRCC	Allog. mDCs laded with autol. tumor cell lysate	none/ Cyclophosphamide; DC pulsed with KLH	20	2 MR, 3 SD
(Wierecky <i>et al.</i> 2006)	mRCC HLA-A*02	Autol. mDCs loaded with two HLA-A*02 MUC1 peptides	pan DR-peptide PADRE, IL-2 (post vaccination period)	20	1 CR, 2 PR, 2 MR, 5 SD
(Bleumer <i>et al.</i> 2007)	metastatic ccRCC HLA-A*02	Autol. mDCs loaded with an HLA-A*02 and HLA-DR restricted CA9 derived peptide	DC pulsed with KLH	6	all PD
(Wei <i>et al.</i> 2007)	mRCC	Autol. mDCs fused with autol. tumor derived cells (Dendritomas)	IL-2	10	1 PR, 3 SD
(Matsumoto <i>et al.</i> 2007)	mRCC	Autol. mDCs loaded with autol. Primary tumor derived cell lysate	DC pulsed with KLH	3	1 SD
(Kim <i>et al.</i> 2007)	mRCC	Autol. mDCs loaded with autol. Primary tumor derived cell lysate	DC pulsed with KLH	9	1 PR, 5 SD
(Berntsen <i>et al.</i> 2008)	mRCC, HLA-A*02 pos or neg	Autol. mDCs loaded with allog. cell line lysate (A*02 neg) or a mix of 9 different hTERT and 11 Survivin derived peptides (A*02 pos)	IL-2, pan DR-peptide PADRE, DC pulsed with KLH (only A*02 neg)	27	13 SD
(Tatsugami <i>et al.</i> 2008)	mRCC	Autol. mDCs loaded with autol. Primary tumor derived cell lysate	IFNa	7	5 SD
(Schwaab <i>et al.</i> 2009)	mRCC	Autol. mDCs loaded with autol. Primary tumor derived cell lysate	IL-2 and IFNa	18	3 CR, 6 PR, 6 SD

*Number of patients treated versus number of patients in control group in brackets. mRCC: metastatic renal cell carcinoma, ccRCC: clear cell renal cell carcinoma, autol.: autologous, allog.: allogenic, mDC: mature dendritic cells, iDC: immature dendritic cells, BCG: Bacille Calmette Guerin, IFA: Incomplete Freund's adjuvant CP: Cyclophosphamide, CR: complete response, PR: partial response, MR: mixed response, OR: objective response, SD: stable disease, PD: progressive disease, OS.: overall survival RFS.: regression free survival, PFS: progression free survival, DFS: disease free survival.

Table 1. Immunotherapeutical studies in RCC

Apart from the tumor vaccination studies mentioned above other approaches involving the transfer of leukocytes such as adoptive T-cell transfer and even stem cell transplantation have been applied in some cases. Allogenic stem cell transplantation has been used successfully for several decades in hematological cancers. Lymphocytes, in particular T-cells of HLA identical donors, are thought to exert a graft-vs-leukaemia effect mainly by recognition of minor histocompatibility antigens on recipient tissues (Bleakley & Riddell 2004) but probably also tumor cell specific antigens (Tykodi *et al.* 2004). The curative or graft-vs-leukaemia effect (GvL) therefore often directly correlates with the appearance of graft vs host disease (GvHD). The use of stem cell transplantation in solid tumors has been shown to provide a similar graft-vs-tumor (GvT) effect in renal cell carcinoma, but also in ovarian (Bay *et al.* 2002) and breast cancer patients (Bishop *et al.* 2004). In the context of solid tumors non-myeloablative regimens have been preferred to avoid the substantial side effects of myeloablation with high dose chemotherapy and irradiation. Engraftment thus does not lead to complete substitution of the host's immune system but rather to a chimeric state incorporating both the recipient's and the donor's hematological system. First studies of non-myeloablative allogenic stem cell transplantation (NST) in 17 mRCC patients showed quite promising results (Childs *et al.* 2000) with partial regression in over 50% including three patients with prolonged complete response. Further studies have been conducted (for a comprehensive overview see (Demirer *et al.* 2008)) with small patient numbers and varying response rates ranging from 0% (Rini *et al.* 2006) to more than 50% (Bregni *et al.* 2002). However, NST is also facing several other difficulties. Besides the need for an HLA matched donor, severe and sometimes fatal complications from transplantation and subsequent GvHD can occur. Furthermore the time from treatment to response can take several months and in order to avoid graft rejection, patients have to be kept in an immunosuppressive state which allows for and might even accelerate rapid disease progression.

Adoptive T cell transfer in RCC has played a rather minor role compared to trials in other immunogenic tumors, especially in melanoma. Initial studies with isolated and *ex vivo* expanded tumor infiltrating lymphocytes (TILs) that have been reinfused into the patient usually in combination with IL-2, showed only modest response (Topalian *et al.* 1988; Kradin *et al.* 1989). In a larger phase III trial involving 160 patients with metastatic RCC, treatment with *ex vivo* expanded TILs and IL-2 showed no benefit compared to IL-2 alone (Figlin *et al.* 1999). Because of these disappointing results and because of the lack of tumor specific T-cell epitopes further use of (antigen specific) adoptive T-cell transfer has been limited although recently the usage of gammadelta T cells in RCC patients has moved into the focus of ongoing research (Bennouna *et al.* 2008; Kobayashi *et al.* 2010). In order to complete the picture of current immunotherapeutic approaches, WX-G250 (Girentuximab) has to be mentioned. This is an antibody developed by Willex that is specifically intended for adjuvant use in non-metastatic RCC patients and is currently undergoing Phase III clinical trials (Reichert 2011). The chimeric IgG1 antibody is directed against Carbonic anhydrase 9, which is a tumor associated antigen expressed by more than 90% of clear cell renal cell carcinoma. Data from a recent Phase I/II trial of Girentuximab in combination with IFN α for metastatic RCC patients have shown good tolerability, safety and also clinical benefit (Siebels *et al.* 2011).

Considering the diversity of different immunotherapeutic approaches, especially in tumor vaccination, the question remains why many of these studies failed or showed rather limited

clinical success, with only a few trials progressing to clinical phase III. In order to answer this question we will first look at the main problem of all immunotherapeutic approaches, namely the ability of a tumor to escape a directed immune response by inducing an immunosuppressive environment. In this context we will also discuss methodological deficiencies and contradictions that have contributed to clinical failure and describe more promising directions for future immunotherapy.

4. Immunosuppression and tumor escape

As mentioned above, renal cell carcinomas, like other tumors, are highly infiltrated by leukocytes and especially T cells (> 60%) mainly of the CD8⁺ rather than the CD4⁺ phenotype. Natural killer cells have also been found to be enriched within the TIL population in some studies at least whereas B cells make up only a minor subset. (Van den Hove, Van Gool et al. 1997). Infiltrating T cells are predominantly of the antigen experienced effector memory type (T_{EM}), and CD8⁺ cells also encompass highly differentiated T_{EMRA} effector cells (Attig *et al.* 2009). Expression of several lymphocyte activation markers such as CD69 or HLA-DR on TILs has been confirmed and oligoclonal expansion of certain TCR-V β regions indicates that a selection of potentially tumor specific T cells has taken place (Angevin *et al.* 1997). Furthermore, after isolation and ex vivo culture T cells are able to express cytokines and show normal cytotoxic activity. However if these TILs show functionality *ex vivo* then why are they not reactive within the tumor microenvironment? Indeed, freshly isolated uncultured TILs often show a reduced capacity of their cytotoxic function (Van den Hove, Van Gool et al. 1997) and also an impaired cytokine production or altered cytokine profile, demonstrating that some kind of immunosuppressive milieu must be present within the tumor microenvironment (Gouttefangeas *et al.* 2007). Defects in T-cell signalling and downregulation of the CD3 ζ -chain, which is necessary for TCR signal transduction into the cell, can be frequently found in T cells isolated from RCC patients (Frey & Monu 2008). Several mechanisms responsible for this locoregional immunosuppression have been discovered within the last decades and only the most important findings will be described here. Some of these mechanisms, such as the activation of T regulatory cells (T_{regs}), have been intentionally developed by evolution to counteract deleterious long term activation of an immune response in order to avoid autoimmune diseases. Others, such as the generation of inhibitory signals have been developed or rather selected within the heterogeneity of tumor cells to escape an existing immune response and thereby provide a selection advantage. Among the latter are immunosuppressive cytokines IL-10 or TGF- β , which are known to inhibit T-cell activation as well as proliferation and can also lead to downregulation of MHC class I molecules (Khong & Restifo 2002; Li *et al.* 2006). In renal cell carcinoma, proangiogenic vascular endothelial growth factor (VEGF), which acts also immunosuppressively by inhibiting DC maturation (Ohm & Carbone 2001) plays a major role because it is usually found highly overexpressed in clear cell RCCs (Rini 2005). Apart from the expression of immunosuppressive factors, tumor cells can also downregulate costimulatory molecules from their surface. Despite the fact that tumor cells are not professional APCs and therefore not supposed to prime T cells, the complete lack of costimulatory molecules such as CD80 (B7.1) or CD86 (B7.2) can lead to a decreased T-cell activation or even induction of T-cell anergy (Jung *et al.* 1999; Lang *et al.* 2000). In this context another B7 family member should be mentioned which, in contrast to the aforementioned, is often found highly upregulated in different types of cancer. B7-H1

(PDL1) is usually expressed on macrophages and provides costimulatory function for T cells. However, due to its high abundance on tumor cells it has a predominantly negative regulatory activity. After binding to its receptor PD1 on activated T cells B7-H1 can downmodulate T cell activation and even induce apoptosis (Dong *et al.* 2002). B7-H1, and more recently another associate of the B7 family with a similar function, B7-H4, have been shown to be expressed in RCC patients and their expression correlated with adverse clinical prognosis (Thompson *et al.* 2005).

In order to escape an already existing specific immune response, cancer cells can either escape by downregulating the antigen or even parts of the HLA presentation machinery. Indeed downregulation of HLA expression can be observed in several cancer types preferentially in late metastasized stages (Marincola *et al.* 2000; Campoli *et al.* 2002). Different mechanisms underlying this process of downregulation or even complete loss of HLA expression have been elucidated from several tumor cell lines and seem to affect nearly all parts of the antigen processing machinery (Seliger *et al.* 2002). To which extent HLA downregulation or loss plays a role in RCC is still controversial. Whereas some publications suggest that HLA downregulation is a frequent event (Romero *et al.* 2006) other more recent data could clearly show that HLA-expression is not diminished but rather upregulated in comparison to benign kidney tissue (Saenz-Lopez *et al.* 2010; Stickel *et al.* 2011).

Tumor cells can also indirectly inhibit an immune response by depriving proliferating immune cells of essential nutrients. Indoleamine-2,3-dioxygenase (IDO) is an enzyme that catalyzes the first and also rate-limiting step in the degradation of the essential amino acid tryptophane. IDO has been found to be nearly ubiquitously expressed in human tumors (Uyttenhove *et al.* 2003). By locally depleting tryptophane, IDO can inhibit the proliferation of TILs and also induce or recruit T_{regs} (Prendergast *et al.* 2009).

CD4⁺ CD25^{high} FoxP3⁺ regulatory T cells have become a major field of investigation in immunotherapy because of their potential to induce tolerance by suppressing (tumor) antigen specific priming of T cells and also T cell effector functions (Zou 2006). T_{regs} are known to accumulate within the microenvironment of different tumors (Woo *et al.* 2001) and higher levels of T_{regs} in the peripheral blood of cancer patients have been detected. In RCC patients a higher frequency of T_{regs} within the tumor and periphery have been described and correlate with an adverse clinical outcome (Liotta *et al.* 2011). A plethora of different mechanisms have been described on how T_{regs} exert their immunosuppressive functions that range from the expression of immunosuppressive cytokines (IL 10, TGFβ) (Taylor *et al.* 2006), induction of IDO and B7-H4 in APCs (Fallarino *et al.* 2003; Sica *et al.* 2003), and consumption of IL-2 (von Boehmer 2005) to direct cell mediated cytotoxicity (Grossman *et al.* 2004). Antigen specific T_{regs} have been described (Wang *et al.* 2004) but after activation, the suppressive activity of CD4⁺ T_{regs} seems to be antigen non-specific affecting T cells of varying specificity (Thornton & Shevach 2000). Another type of immunosuppressive cells are the myeloid derived suppressor cells (MDSCs) which have recently acquired increasing attention (Kusmartsev & Vieweg 2009). MDSCs are a heterogeneous population of progenitor cells of the myeloid lineage. Under healthy conditions these cells rapidly differentiate into mature granulocytes, macrophages and also dendritic cells. However, in patients suffering from different types of cancer that include RCC (Rodriguez *et al.* 2009), these immature cells have been shown to strongly accumulate in peripheral blood and also

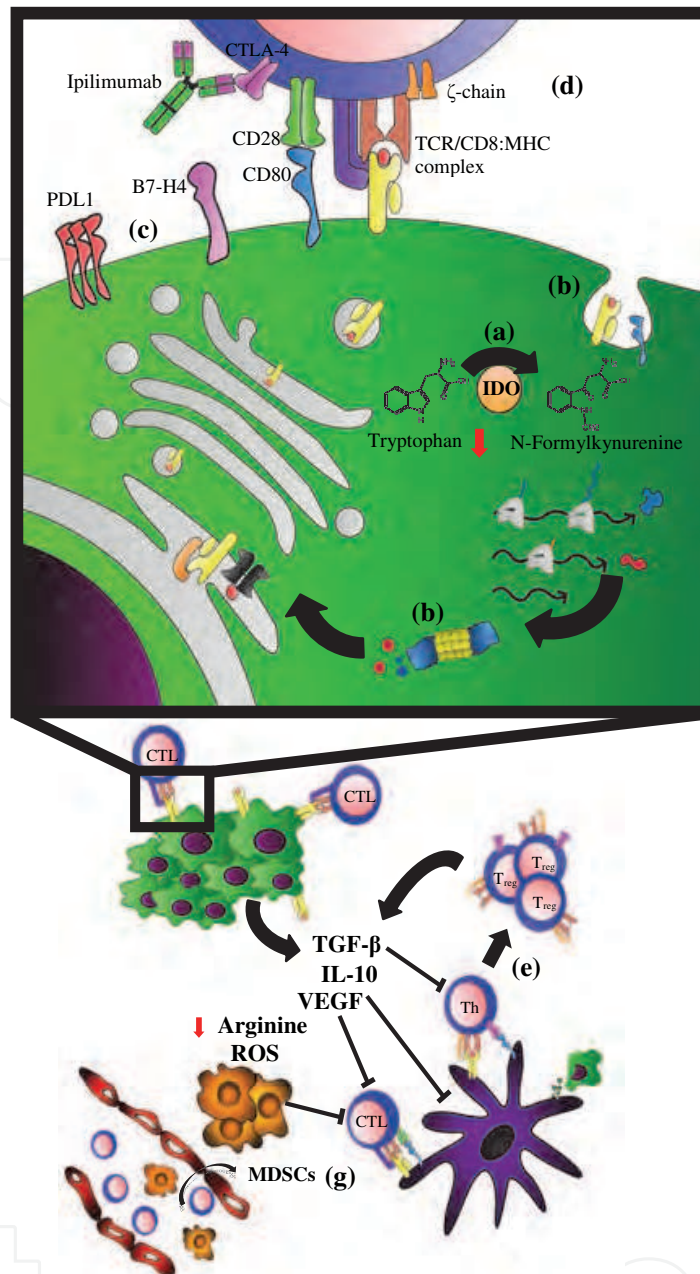


Fig. 1. Immunosuppressive Mechanisms: (a) Overexpression of Indoleamin-2,3-dioxygenase (IDO) leads to reduced extracellular tryptophan levels thereby inhibiting T cell proliferation. (b) Defects in antigen presentation machinery or downregulation of MHC molecules prevent recognition by cytotoxic lymphocytes (CTLs). (c) Aberrant expression of PDL1 or upregulation of coinhibitory molecules B7-H4 decrease costimulation. (d) Downregulation of T-cell receptor (TCR) ζ -chain inhibits intracellular signalling. (e) Secretion of immunosuppressive cytokines by tumor cells or regulatory T cells (T_{reg} s) inhibits activation of T cells (IL-10, TGF- β) and dendritic cells (VEGF). In addition induction of Tregs (f) from the CD4⁺ T helper cell (Th) population is increased. (g) Infiltration of myeloid derived suppressor cells (MDSCs) from the blood lead to production of reactive oxygen species (ROS) and enhanced arginine catabolism through arginase and iNOS thereby further hampering T cell proliferation.

within the tumor microenvironment (Young *et al.* 1997; Nagaraj & Gabrilovich 2010) where they exert potent immunosuppressive effects. Myeloid suppressor cells seem to highly express two enzymes involved in the arginine metabolism: Arginase and inducible nitric oxid synthase (iNOS). These enzymes drive the enhanced catabolism of arginine which subsequently leads to an arginine depletion from the tumor microenvironment thereby strongly inhibiting T-cell proliferation (Ochoa *et al.* 2007; Gabrilovich & Nagaraj 2009). Higher arginase activity in PBMCs from RCC patients could indeed be demonstrated (Zea *et al.* 2005). Other mechanisms of MDSC mediated immunosuppression, such as the production of reactive oxygen species (ROS) *e.g.* peroxynitrite (Kusmartsev *et al.* 2004), the secretion of immunosuppressive cytokine TGF- β (Filipazzi *et al.* 2007) and the induction of other immune suppressive cells such as T_{regs} (Serafini *et al.* 2008) could also be observed.

Despite all these different immunosuppressive cells and the mechanisms discussed (see Figure 1), the infiltration of cancer tissue by leukocytes still remains a reliable marker for patient outcome and correlates positively with clinical prognosis in RCC patients (Pages *et al.* 2010). Galon *et al.* have shown that the presence and composition of the immunological infiltrate is highly predictive for clinical outcome in colon cancer patients and can even be superior to classical TNM staging (Galon *et al.* 2006).

5. Counteracting immunosuppression

In view of all these sophisticated immunosuppressive mechanisms there are doubts that we will ever be able to overcome immunosuppression and tumor escape. In order to generate a long lasting immune response which keeps the immune system up and running, tumor associated antigens and T-cell epitopes represent only a part of the whole picture. Adjuvants are therefore urgently needed to specifically address this difficult task. Adjuvants or „Immunologist’s dirty little secrets“ as Janeway termed them (Janeway 1989) are an active component of most protective vaccines against infectious diseases particularly intended to enhance or modulate a specific immune response. Most of the adjuvants approved for human treatment (Alumn, MPL) were actually developed for passive vaccination of healthy patients against infectious diseases - a setting in which an enormously high safety profile of the adjuvant is clearly necessary. However, considering the highly immunosuppressive environment of late stage tumors in particular, the need for stronger immunostimulatory adjuvants has emerged even if they reduce safety and bear the risk of provoking autoimmunity. In the majority of trials involving RCC patients (Table 1) cytokines such as GM-CSF, IL-2 or IFN α have been used to ensure effective priming of T cells and enhance the immune response. Regardless of toxicity problems considering the systemic application of IL-2 and Interferon α , new studies could show that IL-2 also leads to the induction and stimulation of regulatory T cells (Brandenburg *et al.* 2008). Similarly GM-CSF has been shown to stimulate the recruitment of myeloid derived suppressor cells (Serafini *et al.* 2004) and its beneficial effect in cancer vaccination has been challenged by two randomized vaccination trials in melanoma in which patients that additionally received GM-CSF had an inferior immunologic and patient outcome (Faries *et al.* 2009; Slingluff *et al.* 2009). The use of incomplete Freund’s adjuvant and BCG as adjuvants in cancer immunotherapy appears rather outdated. Nevertheless, BCG is still approved for the treatment of superficial bladder cancer. Metabolizable squalene based emulsions (MF59, AF03) and saponins (Quil-A, ISCOM, QS-2) have replaced mineral oil based formulations like Freund’s adjuvant. The use of live-attenuated bacteria like BCG has been discontinued in favor of molecularly defined

TLR agonists. The discovery that toll like receptors (TLRs) constitute part of the innate immune system and can act as pattern recognition receptors (PRRs) for different pathogen associated molecular patterns (PAMPs) has boosted the search for agonistic TLR ligands. Synthetic TLR ligands can, as their natural counterparts, bind to TLRs on different cell types and subsequently lead to the activation of the cell (*e.g.* maturation, release of proinflammatory cytokines, enhanced antigen presentation or effector function). In theory a combination of different TLR agonists can be used to tailor an immune response to fit individual needs (Adams 2009). For example, TLRs 3, 7/8 and 9 have been shown to lead to the preferential activation of a Th1 response which has been known for a long time to be a prognostic factor in cancer immunotherapy correlating with favourable outcome. Several of these synthetic TLR agonists such as PolyI:C (TLR3 agonist), Resiquimod (TLR7/8 agonist) and CpG (TLR9 agonist) are currently being evaluated in clinical trials (Adams *et al.* 2008; Cheever 2008).

Only very few studies in RCC patients have attempted to address the specific requirements needed to overcome immune escape. Some groups have used cyclophosphamide prior to or during vaccination because it is known that low doses of this chemotherapeutic drug lead to improved immune responses potentially by depleting T_{regs} . (Ghiringhelli *et al.* 2004; Lutsiak *et al.* 2005). Denileukin (Diftitox, Ontak), a recombinant fusion protein of IL-2 and diphtheria toxin has been also used successfully for the same purpose (Atchison *et al.* 2010). However some studies have described severe side effects in conjunction with Denileukin like vision loss and vascular leak syndrome (Park *et al.* 2007; Avarbock *et al.* 2008), treatment failure has also been reported (Welters *et al.* 2009). Recent studies are suggesting that pretreatment with less toxic Sunitinib might have a similar effect on regulatory T cells (Finke *et al.* 2008).

The most promising agents that are currently being developed to overcome tumor induced immunosuppression are immune modulating antibodies that inhibit immune checkpoint controls (Fife & Bluestone 2008). The first antibody with this mode of action (Ipilimumab) has just recently been approved by regulatory authorities in the US and Europe as a monotherapy for the treatment of late stage melanoma. Ipilimumab is directed against cytotoxic T-lymphocyte antigen 4 (CTLA-4), a negative immunoregulatory receptor expressed by activated T cells, in particular T_{regs} . CTLA-4 binds to costimulatory molecules (*e.g.* CD80, CD86) on APCs or tumor cells and thereby directly competes with T cell coactivating molecule CD28. However whereas signalling through CD28 leads to T cell activation CTLA-4 signalling is rather inhibitory and promotes T cell tolerization and anergy. Blocking of CTLA-4 by Ipilimumab consequently leads to an increase in T-cell activating signals resulting in a greatly sustained activation of an immune response. Considering its unspecific mechanism of action it is not surprising that among the different side effects experienced after treatment with Ipilimumab, the induction of autoimmune phenomena was also quite frequent. Nevertheless, in most cases the treatment with corticosteroids was effective and did not interfere with clinical benefit.

Based on this very recent breakthrough, several trials are currently evaluating combinations of Ipilimumab with targeted immunotherapeutic approaches in order to highlight the direction to activated T cells. Another immunomodulating antibody directed against PD1 (MDX-1106) is currently being applied in late phase clinical trials. Interaction of PD1 with its ligand B7-H1, which is broadly overexpressed on tumor cells has already been discussed as an established tumor escape mechanism.

The advent of new immunotherapies has also clearly revealed another problem regarding the kinetics and study endpoints of clinical immunotherapeutic trials (Hoos *et al.* 2010). Evaluation of clinical benefit is usually based on world health organization (WHO) (World Health Organization. 1979) or response evaluation criteria in solid tumors (RECIST) (Therasse *et al.* 2000; Eisenhauer *et al.* 2009). These are criteria that were originally developed for treatment with cytotoxic agents. However, patterns of clinical response may greatly differ in immunotherapeutic trials. Compared to chemotherapeutics, the manifestation of clinical response is often delayed in immunotherapeutic trials and develops after an initial phase of stable disease or even tumor progression. This effect is frequently observed in immunotherapeutic trials and is expressed by a delayed separation of Kaplan-Meier survival curves (Hoos *et al.* 2010). Furthermore, a well-known discrepancy of many immunotherapies that fail to achieve clinical benefit in progression-free survival but do show an objective effect on overall survival can be explained by this effect. In order to accommodate these observations, new immune related response criteria (irRC) have been proposed and are currently being tested for their significance and applicability in immunotherapeutic trials (Wolchok *et al.* 2009).

Another question that remains in this context is the appropriate general setting of an immunotherapeutic approach. Nearly all trials mentioned in Table 1 focus on late stage patients with metastatic RCC that have often not responded to previous therapies and have acquired a resistance to cytokine treatment and targeted therapy. Some authors have suggested that this might be a rather unfavourable setting for testing the efficiency of an immunotherapeutic approach because the immune system has probably lost the battle against the cancer at a much earlier stage and immune suppressive mechanisms may have progressed to an irreversible state. The use of immunotherapies should therefore be tested preferentially in an adjuvant or minimal residual disease setting (Morse *et al.* 2005; Hoos *et al.* 2007).

6. Tumor associated antigens: the good, the bad, and the ugly

The lack of defined tumor specific antigens in RCC can already be deduced from the list of studies undertaken so far. Only few invoke defined antigens, most make use of autologous tumor tissue alone, in combination with adjuvant or after loading on dendritic cells. The use of non-defined antigens has several inherent problems however. First, the quality of the vaccine is critically dependent on the purity of the antigen and tissue material received from surgery is undoubtedly of varying quality. Furthermore, some studies have shown that only apoptotic and not necrotic cell vaccines can induce a regular immune response (Scheffer *et al.* 2003). Second, a mixture of different unknown antigens always has the potential of partially or preferentially inducing tolerogenic T cells or, even worse, inducing autoimmunity against self-antigens. Last but not least, immunomonitoring of patients, which has become a powerful tool in recent immunotherapeutic studies, can only be carried out accurately if the antigen is known in advance. Compared with full length proteins, antigenic peptides have the advantage of simple chemically defined production in GMP quality. This allows for the combination of different antigenic peptides to a multi-epitope vaccine. Thereby the individuality of a patient's immune response is taken into account, indicating that not every patient will develop an equally strong immune response against one and the same antigen. The major disadvantage of defined T-cell epitopes is that they are usually restricted to for one HLA allotype requiring the patient to match to a certain HLA in

order to benefit from the treatment. This further underlines the need for the identification of additional tumor-associated antigen-derived peptides for less common HLA alleles (Klug *et al.* 2009).

How can we now decide whether an identified antigen is also a good vaccination candidate and what are the hallmarks of these antigens? The most important but often carelessly neglected requirement of any good vaccination candidate is the presence of the antigen on the tumor cell. In terms of an immunotherapy this means that the antigen needs to be accessible in order to be recognized by immune cells, preferentially T cells. At first glance this sounds trivial but in fact most studies have made use of antigens that have actually never been found to be presented by MHC molecules and instead emanated from *in vitro* tested or *in silico* predicted T cell epitopes of previously known tumor associated antigens that were shown to be overexpressed within tumor tissue at the mRNA or protein level. In doing so basic principles of immunology are ignored since T cells are not given insight into tumor cells but have to rely on a showcase of peptide antigens presented by MHC molecules. Indeed it could be shown that the gene expression level of a certain protein and presentation of corresponding protein-derived peptides only reveal a very faint correlation (Weinzierl *et al.* 2007).

The second most important requirement is the immunogenicity of the antigen. This is indeed a crucial point, since many of the tumor antigens are derived from self proteins and hence show only weak immunogenicity if at all because of central or peripheral T-cell tolerance. One great exception are unique antigens that arise from gene mutations or fusion proteins which accumulate during the course of tumor development. These neo-antigens are therefore not affected by tolerance mechanisms, either in the thymus during lymphocyte maturation by clonal deletion or in the periphery by anergy induction. Because of the large set of distinct mutations acquired within each tumor not only within different genes, but also at a multitude of locations within the gene, the application of mutated antigens in tumor vaccination will be restricted to a patient individualized approach.

Other frequently examined antigens including cancer testis or differentiation antigens show a rather restricted expression pattern that can in some cases also be considered to be tumor specific. During the course of tumorigenesis cancer cells often acquire epigenetic alterations that lead to the expression of genes (MAGE, NY-ESO-1) which are usually only transcribed during embryonic development and hence remain silenced within adult tissue. Some of these cancer testis antigens (CTA) have, however, also been found to be expressed in thymic epithelium so that central tolerance by deletion of CTA-specific T cells cannot be excluded (Gotter *et al.* 2004). Differentiation antigens are commonly expressed in malignant and normal cells of the same lineage *e.g.* Melan-A, gp100 and TRP1 in melanoma tumor tissue and benign melanocytes. In cases in which the benign tissue expressing the differentiation antigen is dispensable as, for instance, in prostate cancer patients after prostatectomy, differentiation antigens like PSA become highly specific for the tumor. The great majority of potential vaccination antigens is, however, derived from tumor antigens that are rather ubiquitously expressed but show a (high) overexpression on cancer tissue (CA9, Her2/neu, MUC1) (Kessler & Melief 2007). Differentiation and overexpressed antigens are usually subject to central and peripheral tolerance mechanisms. Self tolerance for many antigens, however is not always complete. The prior testing of T-cell immunogenicity for these antigens is therefore absolutely essential.

Another aspect that should be considered before choosing a vaccination antigen is its impact on tumor oncogenicity. Preferentially, antigens should be targeted that are involved in the oncogenic process and hence indispensable for tumor growth and maintenance of the neoplastic state.

Based on similar but not identical criteria, a ranking of potentially suitable tumor associated antigens for clinical use has been published in an initiative from the National Cancer Institute involving different working groups in the US (Cheever *et al.* 2009). They used an analytical hierarchy process generated ranking which further included objective criteria like therapeutic functionality, the expression of the antigen within stem cells, number of patients expressing the antigen, the number of known antigen derived T-cell epitopes and also the cellular localisation of the antigen.

7. Identification of new tumor associated antigens

Having described the hallmarks of optimal tumor associated and specific antigens in the context of an anti-tumor vaccine we would now like to present state-of-the-art technology for the identification of respective antigens with a clear focus on tumor associated HLA ligands. There are several strategies which can be divided into two basic sets: Top-down approaches carried out - by directly analysing the tumor antigens present within or presented by tumor cells, and bottom-up. The latter represent a reverse approach usually starting from the gene level *via* the protein level and subsequently to the HLA ligand level.

7.1 Bottom-up

One of the first approaches developed for the large scale identification of tumor associated antigens is the serological identification of antigens by recombinant expression cloning SEREX (Sahin *et al.* 1995). The method is based on cDNA expression libraries created from tumor cell lines or tissues which are subsequently packaged into lambda-phage vectors that can be used to infect *E. coli* bacteria. During the lytic phase the production of recombinant proteins is induced and clones can be screened for the presence of tumor reactive antibodies using cancer patient sera. Selection, cloning and sequencing of antibody reacting clones allows for the straightforward molecular description of the antigen, as the recombinant proteins are located in the same *E.coli* clone plaque as the respective cDNA (Tureci *et al.* 2005). The major advantage of cDNA expression libraries is that only genes that are actually expressed within the cell/tissue of origin are incorporated and that they can be generated from various sources that include patient derived autologous tumor tissue. SEREX is still a valued, commonly used tool for antigen identification (Wang *et al.* 2009; Kiyamova *et al.* 2010). Several modifications have been incorporated to overcome restrictions for instance regarding the prokaryotic expression system, which does not allow for posttranslational modifications (Kim *et al.* 2007). With the advent of the „omics“ era a comparatively high throughput analysis of differences in the gene expression level could be done with relative ease. Now large databases summarizing these data are publicly available (Edgar *et al.* 2002). New approaches aim at gaining a deeper understanding of the tumor and its genetic basis by sequencing the complete tumor genome. The whole genome approach has profited from the rapid progress in next-generation sequencing techniques over the last few years which is fortunately accompanied by decreasing costs (Wong *et al.* 2011). The complete and differential sequencing of tumor tissue and corresponding normal tissue shows alterations

directly on genomic level that the tumor has acquired during its development (see Fig. 2 blue section). The subset of potential tumor antigens can be assessed by the selection of non-silent mutational events. Further reconciliation with databases of known sequence polymorphisms (dbSNP, (Smigielski *et al.* 2000)) or known mutations in cancer (COSMIC, (Bamford *et al.* 2004)) can help to distinguish tumor driver from passenger mutations.

The bottom-up approach yields proteins without any post-translational modifications. A prerequisite for successful peptide vaccination therapy is the presentation on the tumor cell surface by MHC molecules. *In silico* digestion and HLA binding prediction (Feldhahn *et al.* 2009) of the respective parts can shed light on which of these are putative tumor antigens suitable for further evaluation of immunogenicity in T-cell arrays.

A major determinant for peptide:HLA binding is the steric configuration defined by the MHC molecule and the amino acid sequence of the peptide (Bjorkman *et al.* 1987). The HLA molecule forms a peptide binding groove with prominent binding pockets for individual amino acid side chains. The polymorphism of the HLA alleles results in different polypeptides and therefore different binding pocket properties. This yields characteristic peptide sequence motifs for ligands to bind the HLA molecule. The most conserved positions in these motifs form the anchor residues, whose side chains fit best in the binding pockets for strong interaction.

With sufficient ligandome analysis that takes sequence statistics for the given peptide:HLA molecule pairs into consideration one can generate Position Specific Scoring Matrices (PSSMs). Each position holds higher scores for more frequently occurring amino acids. Summing up the scores for each position, these matrices can be used to estimate the binding capability with an HLA molecule for any given peptide sequence. The SYFPEITHI method uses PSSMs generated from naturally processed HLA ligands from the SYFPEITHI database (Rammensee *et al.* 1999) in an expert system fashion also accounting for given chemical conditions. The assumption of independent contribution of each amino acid to the overall binding affinity is one drawback of PSSM approaches. Non-linear machine learning methods can create prediction models that address this issue with different techniques (support vector machine (SVM) based SVMHC (Dönnes & Kohlbacher 2006), artificial neural network (ANN) training method based NetMHC (Buus *et al.* 2003). Prediction quality for all approaches is heavily dependent on the sampling coverage for an allele-specific HLA ligandome available (discussion of efficiency is out of scope).

There also exist prediction methods for the steps preceding peptide presentation, the proteasomal cleavage of proteins (PAProC (Nussbaum *et al.* 2001), NetChop, (Kesmir *et al.* 2002)) and the TAP transport (TAPPred, (Bhasin & Raghava 2004)). As these steps are very complex and depend on a huge variety of parameters, the results may not adequately reflect the naturally occurring process. The framework for T-cell epitope detection FRED provides easy accession to most prediction methods for MHC binding as well as creating a general infrastructure for the handling of antigen sequence data. It includes the possibility for integrated analysis of protein polymorphisms influences and simultaneous accession of different prediction methods. Analysis pipelines intended for high throughput capability profit immensely from such frameworks. They integrate different algorithms with differing input and output types and therefore provide a flexible means to implement of accelerating the analysis process.

7.2 Top-down

Until now straightforward proteomics approaches have mainly focused on the identification of serum cancer biomarkers rather than target antigens for cancer therapy (Seliger *et al.* 2003). One exception is serological proteome analysis, SERPA, a modified SEREX approach translated to the protein level. In contrast to SEREX, screening with autologous patient sera is not carried out against cDNA expression libraries but against protein lysates separated by two dimensional polyacrylamide gel electrophoresis (2D-PAGE) or directly against protein arrays (Desmetz *et al.* 2009). The comparative full proteome analysis of tumor and benign tissue usually requires the pre-separation of proteins, for example by 2D-PAGE or multidimensional liquid chromatography, due to the overall complexity of the protein lysate. This approach has in the past suffered from low sensitivity and weak reproducibility (Baggerman *et al.* 2005). Identification of tumor associated target antigens has remained rare since standard proteomics approaches often fail to detect low abundant proteins (Joshi *et al.* 2011). Nevertheless proteomics is a rapidly developing field. Taking the steadily increasing mass spectrometric sensitivity (Yates *et al.* 2009), the use of *in vitro* (e.g. chemical modification) or *in vivo* (e.g. SILAC) differential labeling approaches for quantification (Schulze & Usadel 2010) and also MALDI imaging technology (Fournier *et al.* 2008) into account, proteomics will clearly contribute to a greater extent to TAA identification in the future.

In contrast to standard proteomics, HLA ligandomics is a well established, straightforward approach and perfectly suited for the identification of tumor associated HLA ligands (Schirle *et al.* 2000). HLA molecules can be directly isolated from dissected tumors and autologous normal tissue. The method of choice is immunoaffinity chromatography using antibodies specific for different HLA molecules (see table 2) immobilised on a sepharose matrix. Application of a crude tissue lysate leads to the binding of respective HLA molecules which can be subsequently eluted by acid treatment. At the same time, pH shift also leads to the release of bound peptides from the HLA binding grooves. Due to the narrow mass range of these peptide ligands, they can be easily separated from higher molecular weight substances (>10 kDa) by ultra-filtration. Lyophilisation of the filtrate yields a mixture of different HLA derived peptides ready for concomitant separation and analysis using liquid-chromatography coupled mass spectrometry (LC-MS, see Fig. 2a).

Clone	α HLA	Reference
W6/32	A,B,C	(Barnstable <i>et al.</i> 1978)
B1.23.2	B,C	(Rebai & Malissen 1983)
BB7.2	A2	(Parham & Brodsky 1981)
GAP-A3	A3	(Berger <i>et al.</i> 1982)
Spv-L3	DQ	(Spits <i>et al.</i> 1983)
Tü-39	DR, DQ, DP	(Maeda & Hirata 1984)
L243	DR	(Lampson & Levy 1980)
IVD-12	DQ	(Kolstad <i>et al.</i> 1987)

Table 2. HLA directed antibodies

The greatest benefit is that the described procedure yields natural ligands, so that neither the proteasomal cleavage nor intracellular transport and loading onto HLA molecules has to be

determined for the respective peptide. The challenge is the molecular characterisation of the heterogeneous mixture of isolated peptides.

Peptide and protein sequencing can be accomplished via tandem mass spectrometry. This is an advanced technique of proteomics analysis and offers a versatile, high-throughput procedure for investigation of protein samples, including sequence identification. The measurement is conducted by recording the mass-to-charge ratio (m/z) of peptide ions with high sensitivity down to the sub-femtomole level. For better identification a second mass analyzer can be added in tandem, where molecules of selected masses are further fragmented (Roepstorff & Fohlman 1984; Johnson *et al.* 1987) and the resulting mass-to-charge ratios measured (see Table 3). Fragmentation of peptides occurs most prominently at the backbone structure, *i.e.* the peptide bonds concatenating the amino acids. The result of a tandem mass spectrometric analysis is a spectrum of fragment m/z values. The fragments containing the N-terminus of the peptide are called „b-ions“ whereas those containing the C-terminus are called „y-ions“, each enumerated by the number of retained amino acids. *Via* the masses of the fragments the spectra can be annotated with the peptide's sequence.

	b-ions	Mass	y-ions	Mass	
1	(S)	-	YFPEITHI	1019.5197	8
2	SY	251.1026	FPEITHI	856.4563	7
3	SYF	398.1710	PEITHI	709.3879	6
4	SYFP	495.2238	EITHI	612.3352	5
5	SYFPE	624.2664	ITHI	483.2926	4
6	SYFPEI	737.3505	THI	370.2085	3
7	SYFPEIT	838.3981	HI	269.1608	2
8	SYFPEITH	975.4571	I	132.1019	1

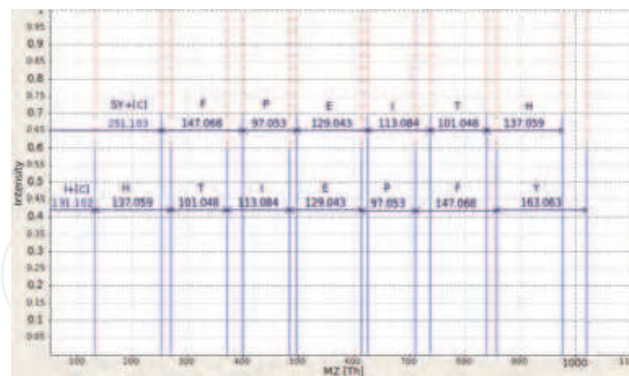


Table 3. Theoretical fragmentation spectrum of SYFPEITHI peptide.

Missing peaks make the spectrum annotation a complex problem. These are due to: technical detection thresholds, complex or incomplete fragmentation, contamination and measurement noise. This can be addressed by computational methods for mass spectrometry proteomics. Spectra can be identified by either algorithmic comparison to a database of known spectra (Eng *et al.* 1994), directly by *de novo* methods (Bertsch *et al.* 2009) that do not depend on databases or by comparison with a sequence database (Perkins *et al.* 1999).

The latter and in most cases very robust method infers theoretical spectra from a set of compatible sequences and matches the experimentally determined masses with those calculated. The method is robust because it is possible to infer a statistical significance evaluation, *i.e.* a score, from the size of the database as well as the number and quality of matches. Another technique to introduce robustness to the results is the use of a false discovery cut off. The introduction of a decoy database (most commonly the inverse input database) allows for calculation of a false discovery rate, defined as the number of false discoveries (from the decoy) over the number of false and correct discoveries (from both databases) given a score. A false discovery rate of 5% discerns a score within one experiment which guarantees that scores equal or better will be false discoveries only by the chance of $p=0.05$ (*i.e.* 5%).

Sophisticated methods for high throughput identification of natural HLA ligands are beginning to emerge. Established proteomics methods are specifically suited for this task. OpenMS (Sturm *et al.* 2008) is an open and flexible framework for proteomics data analysis. As explained, integrated analysis is a major advantage for high throughput oriented analysis. In the case of individualized cancer therapy, different disciplines with diverse methods come together which does not permit a comprehensive framework. One solution for maintaining high throughput capability is a tailored workflow development that integrates analysis tools of different trades. OpenMS/TOPP also comes with tools for workflow development (Kohlbacher *et al.* 2007) for virtually seamless integration of other computational aspects of immunology, such as the *in silico* prediction of HLA presented peptides or database connection and reconciliation containing very different types of data.

7.3 Validation and selection of peptide candidates

The final verification step of identified HLA ligands includes the chemical synthesis of peptides. The tandem mass spectrometric measurement of the synthesized peptide represents a crucial aspect of reliability. As fragmentation patterns define the sequence and are preserved throughout the measurements, the corresponding spectra as well as the retention time of the synthetic and identified peptide will be nearly identical. A putative T-cell epitope needs to be further validated in T-cell priming experiments. For this reason a plethora of protocols has been established to prime naive T cells usually from healthy donors with either natural (*e.g.* dendritic cells) or artificial APCs presenting the synthetic peptide. Proliferation and priming efficiency can subsequently be assayed by a variety of functional tests, *e.g.* enzyme linked immunospot technique (ELISPOT), intracellular cytokine staining (ICS) or tetramer staining.

In order to work reasonably well in a preferably diverse target group, the design of a vaccine also poses the challenge of combining several vaccination candidate peptides to a multi-epitope vaccine. Recently, Toussaint *et al.* (Toussaint & Kohlbacher 2009) proposed a mathematical framework for the selection of an optimal set of peptides for vaccination. Given a set of candidate epitopes, a target population, information on the respective T-cell reactivities to the peptides (or HLA binding affinities), along with other user-defined information to be incorporated in the selection process, the framework efficiently determines an optimal epitope set.

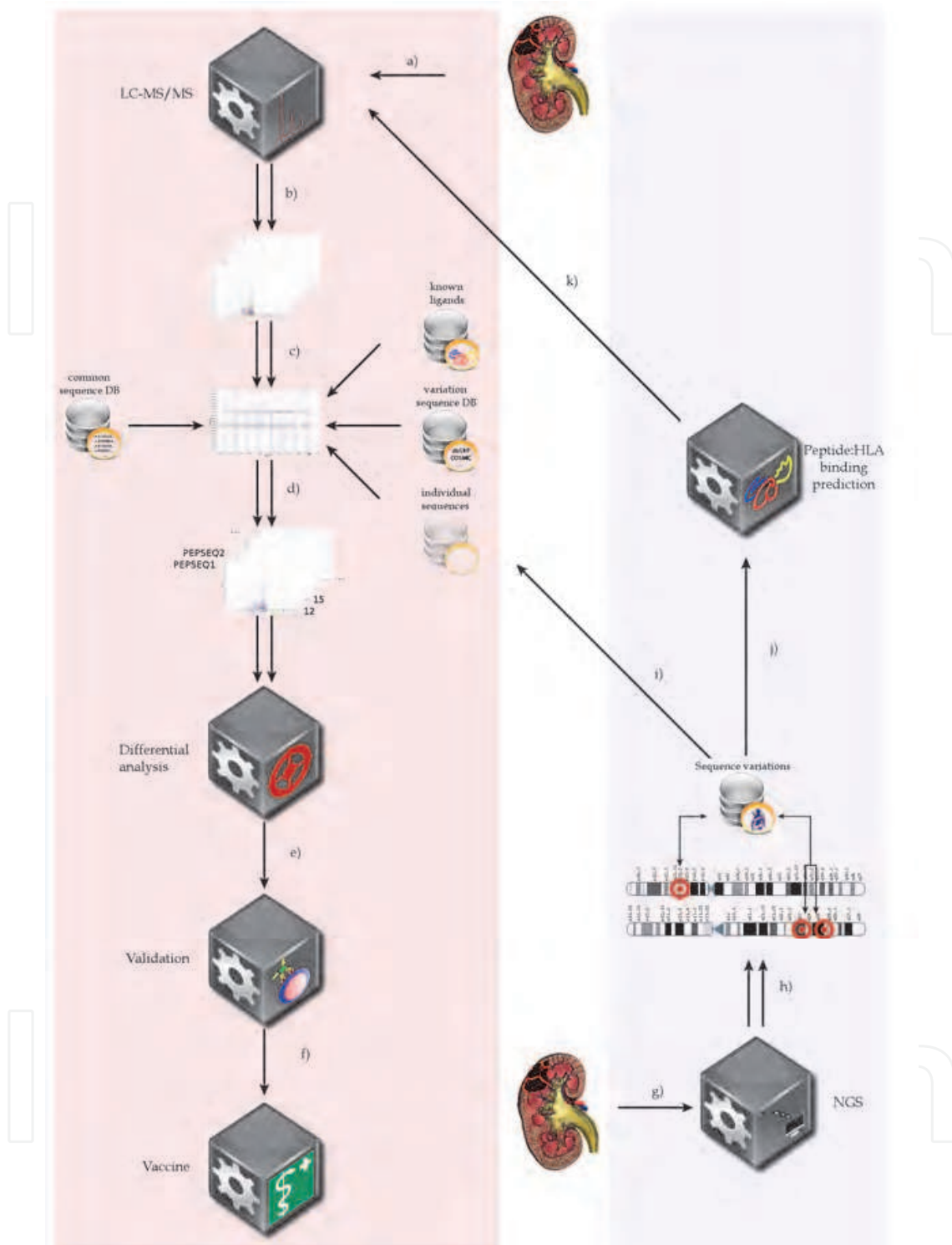


Fig. 2. The workflow for standard HLA ligandome analysis (red) and with mounted bottom-up approach methods (blue) for patient individualisation. a) sample preparation, b) MS analysis, c) peptide identification, d) FDR filter, e) differential analysis, f) HLA ligand validation, g) vaccine design and application, h) genome sequencing, i) variation detection, j) peptide:HLA binding prediction, k) identification of peptides with sequence variation, l) targeted search. Chromosome figures are modified from a screenshot at <http://genome.ucsc.edu> (Kent et al. 2002).

8. Outlook

The therapeutic situation in advanced stage renal cell carcinoma leaves much room for improvement with frequent resistance development to TKIs and still no cure in sight. Despite the lack of convincing clinical success, targeted immunotherapy remains a promising approach. We are only just beginning to understand the complex relationship between the tumor and the surrounding microenvironment. The sophisticated mechanisms of immune evasion that hamper an active immune response are still a major hindrance on the road to success. First steps to overcoming this are by specifically addressing immunosuppression presented through the advent of immune modulatory antibodies such as Ipilimumab. However improvement of targeted immunotherapy is also clearly dependent on the identification of new tumor associated and also specific antigens as well as HLA ligands (Stevanovic 2002). This stresses the need for concise epitope libraries comprising ligands from different tumor entities and benign tissue for various HLA alleles (Krüger *et al.* 2005).

8.1 Targeting the individual patient

The greatest leap forward in the struggle for a successful immunotherapy, at least from the authors' point of view are expected from a patient individualised approach. Tremendous progress in DNA sequencing during the last few years has enabled us to look at the genetic basis of each individual cancer. Comparison of somatic mutations within different tumors has shown that distinct mutational patterns are not only present between different tumor types but also between tumors of the same entity clearly underlining the need for an individualized approach (Ocana & Pandiella 2010).

Combination of whole genome sequencing with HLA ligandome analysis will in future lead to the identification of mutated tumor specific HLA ligands presented on tumor cells. The advanced utilisation of computer-aided high throughput methods enables fast and accurate identification of vaccine candidates. This demonstrates that individualised anti-tumor therapy is also feasible within a certain time frame (first vaccination estimated 5-7 weeks post surgery see Table 4).

Step	Approx. Duration	Section in Figure 2
Genome sequencing	> 2 Weeks	Blue
Ligandome analysis	1-2 weeks	Red
GMP grade peptide synthesis	>2 Weeks	Red
$\Sigma \sim 6$ Weeks		

Table 4. Current time limitations until vaccine availability

The use of mutated HLA ligands should also eliminate the need for time consuming *in vitro* T-cell immunogenicity testing since these antigens are tumor specific neoantigens and thus foreign to the immune system. Differences in the strength of an immune response towards a

certain mutated peptide antigen can, however, not be excluded. Nevertheless, the probability of response failure can be greatly reduced by a combination of several mutated peptides to a multi-epitope vaccine.

Compared to other treatment strategies, immunotherapy aimed at mutated antigens does not necessarily need to target tumor driver mutations, which are usually key to oncogenic properties. Instead, any mutation specific for a certain tumor which is found to be expressed and presented by HLA molecules can be used. Thereby the amount of possible vaccination candidate peptides is greatly increased. The importance of this fact is obvious given that only 2-5 driver mutations among the 1000-10000 somatic mutations found can be unambiguously identified within each tumor (Stratton 2011).

8.2 Targeting the tumor microenvironment

Failure to eradicate cancer by targeting tumor cells exclusively has extended the focus in anticancer therapy during the last few years to include the tumor microenvironment (Kenny *et al.* 2007). Evidence is accumulating to confirm intense crosstalk between tumor cells and their surroundings. Tumor cells not just passively profit from nutritional factors but actively shape their own environment towards their specific needs in a process of coevolution (Polyak *et al.* 2009). Clear cell renal cell carcinoma are a prime example of this since they are usually hypervascularized. This is nearly always the result of a genetic defect in the hypoxia inducible factor 1 signalling cascade leading to secretion of large amounts of proangiogenic VEGF (Sufan *et al.* 2004). Other well established mechanisms of tumor-stroma interaction are the remodelling of the extracellular matrix by tumor-associated macrophages (Mantovani *et al.* 2006), the expression of stimulatory growth factors by tumor associated fibroblasts (Bhowmick *et al.* 2004) and of course the attraction of immune suppressive cells (Bagloli *et al.* 2006). The idea of targeting the cancer's „safe haven“ might therefore not only deprive the tumor from its nutritional basis but hopefully also, at least in part reverse immunosuppression. Targeting the tumor stroma in RCC is already being done: tyrosine kinase inhibitors, which currently represent first line treatment for metastatic RCC, are thought to act primarily on endothelial cells, thereby inhibiting angiogenesis.

Combination therapy of TKIs together with cytokine treatment (Miller & Larkin 2009) as well as TKIs with targeted immunotherapy (Rini *et al.* 2011) are already being tested in clinical trials. But what about targeting the stroma directly with a specific immunotherapy? Stroma cells within tumor cells seem to differ in various aspects from their normal counterparts. Endothelial cells within the tumor show a highly fenestrated chaotic organization and often lack supportive smooth muscle cells as well as a regular basement membrane (Aird 2009). Cancer associated fibroblasts have an activated phenotype with high proliferative capacity, less growth requirements and show a high capacity to recruit endothelial progenitor cells (Orimo & Weinberg 2006; Li *et al.* 2007). Differences between normal and tumor stroma can also be observed on the level of gene expression (Ma *et al.* 2009) and are probably based on different epigenetic alterations (Hu *et al.* 2005). Some groups have reported that tumor associated stroma cells are also subject to clonally selected somatic mutations in tumor suppressor genes (TP53) (Kurose *et al.* 2002) and oncogenes (EGFR) (Weber *et al.* 2005) and further show a high degree of genomic instability (loss of chromosomal heterozygosity) (Moinfar *et al.* 2000). However these results are still controversial and might also be due to technical issues (Polyak, Haviv *et al.* 2009).

Nevertheless, considering the overall changes within tumor associated stroma might render these cells susceptible to an attack by different immune cells. In order to include the targeting of tumor stroma cells into an immunotherapeutic approach, immunological accessible antigens, especially HLA ligands which are exclusively expressed on tumor stroma need to be identified.

8.3 Targeting cancer stem cells

Another upcoming field in cancer therapy is based on the observation that tumor cells within some cancer types at least show a hierarchical organization which is closely resembling that of normal benign tissue. According to the cancer stem cell hypothesis only a rare population of tumor cells termed cancer stem cells (CSC) or tumor initiating cells (TIC) are ultimately responsible for initiating and driving tumor growth. CSCs share some characteristics with normal tissue stem cells including the potential of self renewal, indefinite division, slow replication rate, greatly increased DNA repair capacity and finally the ability to divide asymmetrically, thereby giving rise to new cancer stem cells and rapidly dividing more differentiated tumor cells that make up the bulk of the tumor (Wang & Dick 2005). Evidence for the existence of a tumor initiating cell population was first provided in acute myeloid leukaemia by John Dick and colleagues in the mid 90s (Lapidot *et al.* 1994; Bonnet & Dick 1997). They demonstrated that tumorigenicity of leukaemia cells was much higher in a subpopulation of CD34⁺CD38⁻ tumor cells. Less than 500 cells exhibiting this particular phenotype were sufficient to engraft immunodeficient mice, whereas even a 100-fold higher number of CD34⁺CD38⁺ tumor cells, which constitute the gross of leukaemic cells, could not initiate engraftment. Even more important engraftment of isolated CD34⁺CD38⁻ cells repeated the composition of the original tumor in that CD34⁺CD38⁻ cells also gave rise to the major leukaemic cell population of CD34⁺CD38⁺ cells.

Several years later, the presence of a cancer stem cell population in solid tumors was shown for breast cancer (Al-Hajj *et al.* 2003). Since then, the existence of a CSC-population has been shown for many different types of cancer including brain, prostate, liver, colon and lung (for a review see (Visvader & Lindeman 2008)). In renal cell carcinoma, the existence of a CD105⁺ cancer stem cell population has also been postulated but final proof regarding its existence is still missing (Bussolati *et al.* 2008). The attraction of the cancer stem cell model is in part based on its ability to explain long known but still poorly understood clinical phenomena, the relapse of cancer patients after a period of tumor regression and even absence of detectable lesions. CSC are known to be highly resistant to chemo- and radiotherapy. They can remain quiescent for a prolonged period of time and then restart tumor growth at a distant site leading to metastasis. This bears a fundamental clinical impact for the treatment of cancer patients. As long as CSCs are not effectively killed by a treatment we won't be able to completely eradicate a certain tumor (Clevers 2011). Considering the resistance mechanisms to standard therapeutic approaches, immunotherapy may indeed be the only therapeutic option to directly target these cells. Therefore cancer stem cell specific antigens urgently need to be discovered and their potential for a targeted immunotherapy evaluated.

There is ample evidence that specific immunotherapy will play a very important role in the future not only in RCC but rather for the treatment of different cancer types. The first targeted immunotherapy Provenge (Sipuleucel-T) manufactured by Dendreon has just recently been approved by the FDA for the treatment of advanced prostate cancer. Provenge

consists of an autologous dendritic cell vaccine loaded with a fusion protein of GM-CSF with the prostate specific antigen prostatic acid phosphatase (PAP). Although it is far too early to speak of a triumphal course of this new category of cancer treatment at least a start has been made.

9. Acknowledgements

We specially thank Lynne Yakes for expert proofreading.

10. References

- Adams, S. (2009). Toll-like receptor agonists in cancer therapy. *Immunotherapy*, 1, 6, (Nov, 2009) pp. 949-964
- Adams, S., et al. (2008). Immunization of malignant melanoma patients with full-length NY-ESO-1 protein using TLR7 agonist imiquimod as vaccine adjuvant. *J Immunol*, 181, 1, (Jul 1, 2008) pp. 776-784
- Aird, W. C. (2009). Molecular heterogeneity of tumor endothelium. *Cell Tissue Res*, 335, 1, (Jan, 2009) pp. 271-281
- Al-Hajj, M., et al. (2003). Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci U S A*, 100, 7, (Apr 1, 2003) pp. 3983-3988
- Angevin, E., et al. (1997). Analysis of T-cell immune response in renal cell carcinoma: polarization to type 1-like differentiation pattern, clonal T-cell expansion and tumor-specific cytotoxicity. *Int J Cancer*, 72, 3, (Jul 29, 1997) pp. 431-440
- Antonia, S. J., et al. (2002). Phase I trial of a B7-1 (CD80) gene modified autologous tumor cell vaccine in combination with systemic interleukin-2 in patients with metastatic renal cell carcinoma. *J Urol*, 167, 5, (May, 2002) pp. 1995-2000
- Arroyo, J. C., et al. (2004). Immune response induced in vitro by CD16- and CD16+ monocyte-derived dendritic cells in patients with metastatic renal cell carcinoma treated with dendritic cell vaccines. *J Clin Immunol*, 24, 1, (Jan, 2004) pp. 86-96
- Atchison, E., et al. (2010). A pilot study of denileukin diftitox (DD) in combination with high-dose interleukin-2 (IL-2) for patients with metastatic renal cell carcinoma (RCC). *J Immunother*, 33, 7, (Sep, 2010) pp. 716-722
- Attig, S., et al. (2009). Simultaneous infiltration of polyfunctional effector and suppressor T cells into renal cell carcinomas. *Cancer Res*, 69, 21, (Nov 1, 2009) pp. 8412-8419
- Avarbock, A. B., et al. (2008). Lethal vascular leak syndrome after denileukin diftitox administration to a patient with cutaneous gamma/delta T-cell lymphoma and occult cirrhosis. *Am J Hematol*, 83, 7, (Jul, 2008) pp. 593-595
- Avigan, D., et al. (2004). Fusion cell vaccination of patients with metastatic breast and renal cancer induces immunological and clinical responses. *Clin Cancer Res*, 10, 14, (Jul 15, 2004) pp. 4699-4708
- Avigan, D. E., et al. (2007). Phase I/II study of vaccination with electrofused allogeneic dendritic cells/autologous tumor-derived cells in patients with stage IV renal cell carcinoma. *J Immunother*, 30, 7, (Oct, 2007) pp. 749-761
- Azuma, T., et al. (2002). Dendritic cell immunotherapy for patients with metastatic renal cell carcinoma: University of Tokyo experience. *Int J Urol*, 9, 6, (Jun, 2002) pp. 340-346

- Baggerman, G., *et al.* (2005). Gel-based versus gel-free proteomics: a review. *Comb Chem High Throughput Screen*, 8, 8, (Dec, 2005) pp. 669-677
- Bagloli, C. J., *et al.* (2006). More than structural cells, fibroblasts create and orchestrate the tumor microenvironment. *Immunol Invest*, 35, pp. 297-325
- Bamford, S., *et al.* (2004). The COSMIC (Catalogue of Somatic Mutations in Cancer) database and website. *Br J Cancer*, 91, 2, (Jul, 2004) pp. 355--358
- Barbuto, J. A., *et al.* (2004). Dendritic cell-tumor cell hybrid vaccination for metastatic cancer. *Cancer Immunol Immunother*, 53, 12, (Dec, 2004) pp. 1111-1118
- Barnstable, C. J., *et al.* (1978). Production of monoclonal antibodies to group A erythrocytes, HLA and other human cell surface antigens-new tools for genetic analysis. *Cell*, 14, 1, (May, 1978) pp. 9-20
- Bay, J. O., *et al.* (2002). Allogeneic hematopoietic stem cell transplantation in ovarian carcinoma: results of five patients. *Bone Marrow Transplant*, 30, 2, (Jul, 2002) pp. 95-102
- Bennouna, J., *et al.* (2008). Phase-I study of Innacell gammadelta, an autologous cell-therapy product highly enriched in gamma9delta2 T lymphocytes, in combination with IL-2, in patients with metastatic renal cell carcinoma. *Cancer Immunol Immunother*, 57, 11, (Nov, 2008) pp. 1599-1609
- Berger, A. E., *et al.* (1982). Monoclonal antibody to HLA-A3. *Hybridoma*, pp. 87-90
- Berntsen, A., *et al.* (2008). Therapeutic dendritic cell vaccination of patients with metastatic renal cell carcinoma: a clinical phase 1/2 trial. *J Immunother*, 31, 8, (Oct, 2008) pp. 771-780
- Bertsch, A., *et al.* (2009). De novo peptide sequencing by tandem MS using complementary CID and electron transfer dissociation. *Electrophoresis*, 30, 21, (Nov, 2009) pp. 3736--3747
- Bhasin, M. & G. P. S. Raghava (2004). Analysis and prediction of affinity of TAP binding peptides using cascade SVM. *Protein Sci*, 13, 3, (Mar, 2004) pp. 596--607
- Bhowmick, N. A., *et al.* (2004). Stromal fibroblasts in cancer initiation and progression. *Nature*, 432, 7015, (Nov 18, 2004) pp. 332-337
- Bishop, M. R., *et al.* (2004). Allogeneic lymphocytes induce tumor regression of advanced metastatic breast cancer. *J Clin Oncol*, 22, 19, (Oct 1, 2004) pp. 3886-3892
- Bjorkman, P. J., *et al.* (1987). Structure of the human class I histocompatibility antigen, HLA-A2. *Nature*, 329, 6139, (Oct, 1987) pp. 506-512
- Bleakley, M. & S. R. Riddell (2004). Molecules and mechanisms of the graft-versus-leukaemia effect. *Nat Rev Cancer*, 4, 5, (May, 2004) pp. 371-380
- Bleumer, I., *et al.* (2007). Preliminary analysis of patients with progressive renal cell carcinoma vaccinated with CA9-peptide-pulsed mature dendritic cells. *J Immunother*, 30, 1, (Jan, 2007) pp. 116-122
- Bonnet, D. & J. E. Dick (1997). Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat Med*, 3, 7, (Jul, 1997) pp. 730-737
- Brandenburg, S., *et al.* (2008). IL-2 induces in vivo suppression by CD4(+)CD25(+)Foxp3(+) regulatory T cells. *Eur J Immunol*, 38, 6, (Jun, 2008) pp. 1643-1653

- Bregni, M., *et al.* (2002). Nonmyeloablative conditioning followed by hematopoietic cell allografting and donor lymphocyte infusions for patients with metastatic renal and breast cancer. *Blood*, 99, 11, (Jun 1, 2002) pp. 4234-4236
- Buchner, A., *et al.* (2010). Phase 1 trial of allogeneic gene-modified tumor cell vaccine RCC-26/CD80/IL-2 in patients with metastatic renal cell carcinoma. *Hum Gene Ther*, 21, 3, (Mar, 2010) pp. 285-297
- Burnet, F. M. (1967). Immunological aspects of malignant disease. *Lancet*, 1, 7501, (Jun 3, 1967) pp. 1171-1174
- Bussolati, B., *et al.* (2008). Identification of a tumor-initiating stem cell population in human renal carcinomas. *FASEB J*, 22, 10, (Oct, 2008) pp. 3696-3705
- Buus, S., *et al.* (2003). Sensitive quantitative predictions of peptide-MHC binding by a 'Query by Committee' artificial neural network approach. *Tissue Antigens*, 62, 5, (Nov, 2003) pp. 378-384
- Campoli, M., *et al.* (2002). HLA class I antigen loss, tumor immune escape and immune selection. *Vaccine*, 20 Suppl 4, (Dec 19, 2002) pp. A40-45
- Cheever, M. A. (2008). Twelve immunotherapy drugs that could cure cancers. *Immunol Rev*, 222, (Apr, 2008) pp. 357-368
- Cheever, M. A., *et al.* (2009). The prioritization of cancer antigens: a national cancer institute pilot project for the acceleration of translational research. *Clin Cancer Res*, 15, 17, (Sep 1, 2009) pp. 5323-5337
- Childs, R., *et al.* (2000). Regression of metastatic renal-cell carcinoma after nonmyeloablative allogeneic peripheral-blood stem-cell transplantation. *N Engl J Med*, 343, 11, (Sep 14, 2000) pp. 750-758
- Clevers, H. (2011). The cancer stem cell: premises, promises and challenges. *Nat Med*, 17, 3, (Mar, 2011) pp. 313-319
- Coley, W. B. (1893). The treatment of malignant tumors by repeated inoculations of erysipelas. With a report of ten original cases. *Am. J. Med. Sci.*, 105, (Jan, 1893) pp. 487-511
- Dannull, J., *et al.* (2005). Enhancement of vaccine-mediated antitumor immunity in cancer patients after depletion of regulatory T cells. *J Clin Invest*, 115, 12, (Dec, 2005) pp. 3623-3633
- Demirer, T., *et al.* (2008). Transplantation of allogeneic hematopoietic stem cells: an emerging treatment modality for solid tumors. *Nat Clin Pract Oncol*, 5, 5, (May, 2008) pp. 256-267
- Desmetz, C., *et al.* (2009). Humoral response to cancer as a tool for biomarker discovery. *J Proteomics*, 72, 6, (Aug 20, 2009) pp. 982-988
- Dillman, R., *et al.* (2004). Autologous tumor cell line-derived vaccine for patient-specific treatment of advanced renal cell carcinoma. *Cancer Biother Radiopharm*, 19, 5, (Oct, 2004) pp. 570-580
- Dong, H., *et al.* (2002). Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. *Nat Med*, 8, 8, (Aug, 2002) pp. 793-800
- Dönnes, P. & O. Kohlbacher (2006). SVMHC: a server for prediction of MHC-binding peptides. *Nucleic Acids Res*, 34, Web Server issue, (Jul, 2006) pp. 194-197

- Dudek, A. Z., *et al.* (2008). Autologous large multivalent immunogen vaccine in patients with metastatic melanoma and renal cell carcinoma. *Am J Clin Oncol*, 31, 2, (Apr, 2008) pp. 173-181
- Edgar, R., *et al.* (2002). Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. *Nucleic Acids Res*, 30, 1, (Jan 1, 2002) pp. 207-210
- Ehrlich, P. (1909). Über den jetzigen Stand der Karzinomforschung. *Ned. Tijdschr. Geneesk.*, 5, pp. 273-290
- Eisenhauer, E. A., *et al.* (2009). New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer*, 45, 2, (Jan, 2009) pp. 228-247
- Eng, J. K., *et al.* (1994). An approach to correlate tandem mass spectral data of peptides with amino acid sequences in a protein database. *Journal of the American Society for Mass Spectrometry*, 5, 11, (November, 1994) pp. 976--989
- Fallarino, F., *et al.* (2003). Modulation of tryptophan catabolism by regulatory T cells. *Nat Immunol*, 4, 12, (Dec, 2003) pp. 1206-1212
- Faries, M. B., *et al.* (2009). Effect of granulocyte/macrophage colony-stimulating factor on vaccination with an allogeneic whole-cell melanoma vaccine. *Clin Cancer Res*, 15, 22, (Nov 15, 2009) pp. 7029-7035
- Feldhahn, M., *et al.* (2009). FRED--a framework for T-cell epitope detection. *Bioinformatics*, 25, 20, (Oct, 2009) pp. 2758--2759
- Ferlay, J., *et al.* (2010). Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer*, 127, 12, (Dec 15, 2010) pp. 2893-2917
- Fife, B. T. & J. A. Bluestone (2008). Control of peripheral T-cell tolerance and autoimmunity via the CTLA-4 and PD-1 pathways. *Immunol Rev*, 224, (Aug, 2008) pp. 166-182
- Figdor, C. G., *et al.* (2004). Dendritic cell immunotherapy: mapping the way. *Nat Med*, 10, 5, (May, 2004) pp. 475-480
- Figlin, R. A., *et al.* (1999). Multicenter, randomized, phase III trial of CD8(+) tumor-infiltrating lymphocytes in combination with recombinant interleukin-2 in metastatic renal cell carcinoma. *J Clin Oncol*, 17, 8, (Aug, 1999) pp. 2521-2529
- Filipazzi, P., *et al.* (2007). Identification of a new subset of myeloid suppressor cells in peripheral blood of melanoma patients with modulation by a granulocyte-macrophage colony-stimulation factor-based antitumor vaccine. *J Clin Oncol*, 25, 18, (Jun 20, 2007) pp. 2546-2553
- Finke, J. H., *et al.* (2008). Sunitinib reverses type-1 immune suppression and decreases T-regulatory cells in renal cell carcinoma patients. *Clin Cancer Res*, 14, 20, (Oct 15, 2008) pp. 6674-6682
- Fishman, M., *et al.* (2008). Phase II trial of B7-1 (CD-86) transduced, cultured autologous tumor cell vaccine plus subcutaneous interleukin-2 for treatment of stage IV renal cell carcinoma. *J Immunother*, 31, 1, (Jan, 2008) pp. 72-80
- Fournier, I., *et al.* (2008). Tissue imaging using MALDI-MS: a new frontier of histopathology proteomics. *Expert Rev Proteomics*, 5, 3, (Jun, 2008) pp. 413-424
- Frey, A. B. & N. Monu (2008). Signaling defects in anti-tumor T cells. *Immunol Rev*, 222, (Apr, 2008) pp. 192-205
- Gabrilovich, D. I. & S. Nagaraj (2009). Myeloid-derived suppressor cells as regulators of the immune system. *Nat Rev Immunol*, 9, 3, (Mar, 2009) pp. 162-174

- Galligioni, E., et al. (1996). Adjuvant immunotherapy treatment of renal carcinoma patients with autologous tumor cells and bacillus Calmette-Guerin: five-year results of a prospective randomized study. *Cancer*, 77, 12, (Jun 15, 1996) pp. 2560-2566
- Galon, J., et al. (2006). Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science*, 313, 5795, (Sep 29, 2006) pp. 1960-1964
- Ghiringhelli, F., et al. (2004). CD4+CD25+ regulatory T cells suppress tumor immunity but are sensitive to cyclophosphamide which allows immunotherapy of established tumors to be curative. *Eur J Immunol*, 34, 2, (Feb, 2004) pp. 336-344
- Gitlitz, B. J., et al. (2003). A pilot trial of tumor lysate-loaded dendritic cells for the treatment of metastatic renal cell carcinoma. *J Immunother*, 26, 5, (Sep-Oct, 2003) pp. 412-419
- Gotter, J., et al. (2004). Medullary epithelial cells of the human thymus express a highly diverse selection of tissue-specific genes colocalized in chromosomal clusters. *J Exp Med*, 199, 2, (Jan 19, 2004) pp. 155-166
- Gouttefangeas, C., et al. (2007). Immunotherapy of renal cell carcinoma. *Cancer Immunol Immunother*, 56, 1, (Jan, 2007) pp. 117-128
- Grossman, W. J., et al. (2004). Human T regulatory cells can use the perforin pathway to cause autologous target cell death. *Immunity*, 21, 4, (Oct, 2004) pp. 589-601
- Holtl, L., et al. (2005). Allogeneic dendritic cell vaccination against metastatic renal cell carcinoma with or without cyclophosphamide. *Cancer Immunol Immunother*, 54, 7, (Jul, 2005) pp. 663-670
- Holtl, L., et al. (2002). Immunotherapy of metastatic renal cell carcinoma with tumor lysate-pulsed autologous dendritic cells. *Clin Cancer Res*, 8, 11, (Nov, 2002) pp. 3369-3376
- Hoos, A., et al. (2010). Improved endpoints for cancer immunotherapy trials. *J Natl Cancer Inst*, 102, 18, (Sep 22, 2010) pp. 1388-1397
- Hoos, A., et al. (2010). Development of ipilimumab: contribution to a new paradigm for cancer immunotherapy. *Semin Oncol*, 37, 5, (Oct, 2010) pp. 533-546
- Hoos, A., et al. (2007). A clinical development paradigm for cancer vaccines and related biologics. *J Immunother*, 30, 1, (Jan, 2007) pp. 1-15
- Hu, M., et al. (2005). Distinct epigenetic changes in the stromal cells of breast cancers. *Nat Genet*, 37, 8, (Aug, 2005) pp. 899-905
- Iiyama, T., et al. (2007). WT1 (Wilms' tumor 1) peptide immunotherapy for renal cell carcinoma. *Microbiol Immunol*, 51, 5, pp. 519-530
- Janeway, C. A., Jr. (1989). Approaching the asymptote? Evolution and revolution in immunology. *Cold Spring Harb Symp Quant Biol*, 54 Pt 1, 1989) pp. 1-13
- Jocham, D., et al. (2004). Adjuvant autologous renal tumour cell vaccine and risk of tumour progression in patients with renal-cell carcinoma after radical nephrectomy: phase III, randomised controlled trial. *Lancet*, 363, 9409, (Feb 21, 2004) pp. 594-599
- Johnson, R. S., et al. (1987). Novel fragmentation process of peptides by collision-induced decomposition in a tandem mass spectrometer: differentiation of leucine and isoleucine. *Anal Chem*, 59, 21, (Nov, 1987) pp. 2621--2625
- Jonasch, E., et al. (2008). Vaccination of metastatic renal cell carcinoma patients with autologous tumour-derived vitespen vaccine: clinical findings. *Br J Cancer*, 98, 8, (Apr 22, 2008) pp. 1336-1341
- Joshi, S., et al. (2011). Oncoproteomics. *Clin Chim Acta*, 412, 3-4, (Jan 30, 2011) pp. 217-226

- Jung, D., *et al.* (1999). Gene transfer of the Co-stimulatory molecules B7-1 and B7-2 enhances the immunogenicity of human renal cell carcinoma to a different extent. *Scand J Immunol*, 50, 3, (Sep, 1999) pp. 242-249
- Kenny, P. A., *et al.* (2007). Targeting the tumor microenvironment. *Front Biosci*, 12, 2007) pp. 3468-3474
- Kent, W. J., *et al.* (2002). The human genome browser at UCSC. *Genome Res*, 12, 6, (Jun, 2002) pp. 996--1006
- Kesmir, C., *et al.* (2002). Prediction of proteasome cleavage motifs by neural networks. *Protein Eng*, 15, 4, (Apr, 2002) pp. 287-296
- Kessler, J. H. & C. J. Melief (2007). Identification of T-cell epitopes for cancer immunotherapy. *Leukemia*, 21, 9, (Sep, 2007) pp. 1859-1874
- Khong, H. T. & N. P. Restifo (2002). Natural selection of tumor variants in the generation of "tumor escape" phenotypes. *Nat Immunol*, 3, 11, (Nov, 2002) pp. 999-1005
- Kim, J. H., *et al.* (2007). Phase I/II study of immunotherapy using autologous tumor lysate-pulsed dendritic cells in patients with metastatic renal cell carcinoma. *Clin Immunol*, 125, 3, (Dec, 2007) pp. 257-267
- Kim, M. S., *et al.* (2007). Optimized serological isolation of lung-cancer-associated antigens from a yeast surface-expressed cDNA library. *J Microbiol Biotechnol*, 17, 6, (Jun, 2007) pp. 993-1001
- Kiyamova, R., *et al.* (2010). Identification of tumor-associated antigens from medullary breast carcinoma by a modified SEREX approach. *Mol Biotechnol*, 46, 2, (Oct, 2010) pp. 105-112
- Klug, F., *et al.* (2009). Characterization of MHC ligands for peptide based tumor vaccination. *Curr Pharm Des*, 15, 28, 2009) pp. 3221-3236
- Kobayashi, H., *et al.* (2010). Complete remission of lung metastasis following adoptive immunotherapy using activated autologous gammadelta T-cells in a patient with renal cell carcinoma. *Anticancer Res*, 30, 2, (Feb, 2010) pp. 575-579
- Kohlbacher, O., *et al.* (2007). TOPP--the OpenMS proteomics pipeline. *Bioinformatics*, 23, 2, (Jan, 2007) pp. e191--e197
- Kolstad, A., *et al.* (1987). A human-human hybridoma antibody (TrB12) defining subgroups of HLA-DQw1 and -DQw3. *Hum Immunol*, 20, 3, (Nov, 1987) pp. 219-231
- Kradin, R. L., *et al.* (1989). Tumour-infiltrating lymphocytes and interleukin-2 in treatment of advanced cancer. *Lancet*, 1, 8638, (Mar 18, 1989) pp. 577-580
- Krüger, T., *et al.* (2005). Lessons to be learned from primary renal cell carcinomas: novel tumor antigens and HLA ligands for immunotherapy. *Cancer Immunol Immunother*, 54, 9, (Sep, 2005) pp. 826-836
- Kurose, K., *et al.* (2002). Frequent somatic mutations in PTEN and TP53 are mutually exclusive in the stroma of breast carcinomas. *Nat Genet*, 32, 3, (Nov, 2002) pp. 355-357
- Kusmartsev, S., *et al.* (2004). Antigen-specific inhibition of CD8+ T cell response by immature myeloid cells in cancer is mediated by reactive oxygen species. *J Immunol*, 172, 2, (Jan 15, 2004) pp. 989-999
- Kusmartsev, S. & J. Vieweg (2009). Enhancing the efficacy of cancer vaccines in urologic oncology: new directions. *Nat Rev Urol*, 6, 10, (Oct, 2009) pp. 540-549

- Lampson, L. A. & R. Levy (1980). Two populations of Ia-like molecules on a human B cell line. *J Immunol*, 125, 1, (Jul, 1980) pp. 293-299
- Lang, S., *et al.* (2000). B7.1 on human carcinomas: costimulation of T cells and enhanced tumor-induced T-cell death. *Cell Immunol*, 201, 2, (May 1, 2000) pp. 132-143
- Lapidot, T., *et al.* (1994). A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. *Nature*, 367, 6464, (Feb 17, 1994) pp. 645-648
- Lawrence, H. S., (1959). *Cellular and humoral aspects of the hypersensitive states*, P.B. Hoeber, ISBN, New York, USA
- Li, H., *et al.* (2007). Tumor microenvironment: the role of the tumor stroma in cancer. *J Cell Biochem*, 101, 4, (Jul 1, 2007) pp. 805-815
- Li, M. O., *et al.* (2006). Transforming growth factor-beta regulation of immune responses. *Annu Rev Immunol*, 24, pp. 99-146
- Liotta, F., *et al.* (2011). Frequency of regulatory T cells in peripheral blood and in tumour-infiltrating lymphocytes correlates with poor prognosis in renal cell carcinoma. *BJU Int*, 107, 9, (May, 2011) pp. 1500-1506
- Lokich, J. (1997). Spontaneous regression of metastatic renal cancer. Case report and literature review. *Am J Clin Oncol*, 20, 4, (Aug, 1997) pp. 416-418
- Lutsiak, M. E., *et al.* (2005). Inhibition of CD4(+)25+ T regulatory cell function implicated in enhanced immune response by low-dose cyclophosphamide. *Blood*, 105, 7, (Apr 1, 2005) pp. 2862-2868
- Ma, X. J., *et al.* (2009). Gene expression profiling of the tumor microenvironment during breast cancer progression. *Breast Cancer Res*, 11, 1, (2009) pp. R7
- Maeda, H. & R. Hirata (1984). Separation of four class II molecules from DR2 and DRw6 homozygous B lymphoid cell lines. *Immunogenetics*, 20, 6, pp. 639-647
- Maleckar, J. R. & L. A. Sherman (1987). The composition of the T cell receptor repertoire in nude mice. *J Immunol*, 138, 11, (Jun 1, 1987) pp. 3873-3876
- Mantovani, A., *et al.* (2006). Role of tumor-associated macrophages in tumor progression and invasion. *Cancer Metastasis Rev*, 25, 3, (Sep, 2006) pp. 315-322
- Marincola, F. M., *et al.* (2000). Escape of human solid tumors from T-cell recognition: molecular mechanisms and functional significance. *Adv Immunol*, 74, (2000) pp. 181-273
- Marten, A., *et al.* (2002). Therapeutic vaccination against metastatic renal cell carcinoma by autologous dendritic cells: preclinical results and outcome of a first clinical phase I/II trial. *Cancer Immunol Immunother*, 51, 11-12, (Dec, 2002) pp. 637-644
- Marten, A., *et al.* (2003). Allogeneic dendritic cells fused with tumor cells: preclinical results and outcome of a clinical phase I/II trial in patients with metastatic renal cell carcinoma. *Hum Gene Ther*, 14, 5, (Mar 20, 2003) pp. 483-494
- Marten, A., *et al.* (2006). Telomerase-pulsed dendritic cells: preclinical results and outcome of a clinical phase I/II trial in patients with metastatic renal cell carcinoma. *Ger Med Sci*, 4, (2006) pp. Doc02
- Matsumoto, A., *et al.* (2007). Immunotherapy against metastatic renal cell carcinoma with mature dendritic cells. *Int J Urol*, 14, 4, (Apr, 2007) pp. 277-283
- May, M., *et al.* (2009). [Adjuvant autologous tumour cell vaccination in patients with renal cell carcinoma. Overall survival analysis with a follow-up period in excess of more than 10 years]. *Urologe A*, 48, 9, (Sep, 2009) pp. 1075-1083

- Miller, R. E. & J. M. Larkin (2009). Combination systemic therapy for advanced renal cell carcinoma. *Oncologist*, 14, 12, (Dec, 2009) pp. 1218-1224
- Moinfar, F., et al. (2000). Concurrent and independent genetic alterations in the stromal and epithelial cells of mammary carcinoma: implications for tumorigenesis. *Cancer Res*, 60, 9, (May 1, 2000) pp. 2562-2566
- Moiseyenko, V. M., et al. (2005). Phase I/II trial of gene therapy with autologous tumor cells modified with tag7/PGRP-S gene in patients with disseminated solid tumors: miscellaneous tumors. *Ann Oncol*, 16, 1, (Jan, 2005) pp. 162-168
- Morse, M. A., et al. (2005). Recent developments in therapeutic cancer vaccines. *Nat Clin Pract Oncol*, 2, 2, (Feb, 2005) pp. 108-113
- Nagaraj, S. & D. I. Gabrilovich (2010). Myeloid-derived suppressor cells in human cancer. *Cancer J*, 16, 4, (Jul-Aug, 2010) pp. 348-353
- Negrier, S., et al. (1998). Recombinant human interleukin-2, recombinant human interferon alfa-2a, or both in metastatic renal-cell carcinoma. Groupe Francais d'Immunotherapie. *N Engl J Med*, 338, 18, (Apr 30, 1998) pp. 1272-1278
- Nussbaum, A. K., et al. (2001). PProC: a prediction algorithm for proteasomal cleavages available on the WWW. *Immunogenetics*, 53, 2, (Mar, 2001) pp. 87-94
- Ocana, A. & A. Pandiella (2010). Personalized therapies in the cancer "omics" era. *Mol Cancer*, 9, 2010) pp. 202
- Ochoa, A. C., et al. (2007). Arginase, prostaglandins, and myeloid-derived suppressor cells in renal cell carcinoma. *Clin Cancer Res*, 13, 2 Pt 2, (Jan 15, 2007) pp. 721s-726s
- Ohm, J. E. & D. P. Carbone (2001). VEGF as a mediator of tumor-associated immunodeficiency. *Immunol Res*, 23, 2-3, 2001) pp. 263-272
- Oosterwijk-Wakka, J. C., et al. (2002). Vaccination of patients with metastatic renal cell carcinoma with autologous dendritic cells pulsed with autologous tumor antigens in combination with interleukin-2: a phase 1 study. *J Immunother*, 25, 6, (Nov-Dec, 2002) pp. 500-508
- Orimo, A. & R. A. Weinberg (2006). Stromal fibroblasts in cancer: a novel tumor-promoting cell type. *Cell Cycle*, 5, 15, (Aug, 2006) pp. 1597-1601
- Pages, F., et al. (2010). Immune infiltration in human tumors: a prognostic factor that should not be ignored. *Oncogene*, 29, 8, (Feb 25, 2010) pp. 1093-1102
- Pandha, H. S., et al. (2004). Dendritic cell immunotherapy for urological cancers using cryopreserved allogeneic tumour lysate-pulsed cells: a phase I/II study. *BJU Int*, 94, 3, (Aug, 2004) pp. 412-418
- Parham, P. & F. M. Brodsky (1981). Partial purification and some properties of BB7.2. A cytotoxic monoclonal antibody with specificity for HLA-A2 and a variant of HLA-A28. *Hum Immunol*, 3, 4, (Dec, 1981) pp. 277-299
- Parish, C. R. (2003). Cancer immunotherapy: the past, the present and the future. *Immunol Cell Biol*, 81, 2, (Apr, 2003) pp. 106-113
- Park, M., et al. (2007). Vision loss following denileukin diftitox treatment: a case report of possible posterior ischemic optic neuropathy. *Leuk Lymphoma*, 48, 4, (Apr, 2007) pp. 808-811
- Perkins, D. N., et al. (1999). Probability-based protein identification by searching sequence databases using mass spectrometry data. *Electrophoresis*, 20, 18, (Dec, 1999) pp. 3551-3567

- Pizza, G., *et al.* (2004). Allogeneic gene-modified tumour cells in metastatic kidney cancer. Report II. *Folia Biol (Praha)*, 50, 6, pp. 175-183
- Polyak, K., *et al.* (2009). Co-evolution of tumor cells and their microenvironment. *Trends Genet*, 25, 1, (Jan, 2009) pp. 30-38
- Prendergast, G. C., *et al.* (2009). IDO recruits Tregs in melanoma. *Cell Cycle*, 8, 12, (Jun 15, 2009) pp. 1818-1819
- Rammensee, H., *et al.* (1999). SYFPEITHI: database for MHC ligands and peptide motifs. *Immunogenetics*, 50, 3-4, (Nov, 1999) pp. 213-219
- Rebai, N. & B. Malissen (1983). Structural and genetic analyses of HLA class I molecules using monoclonal xenoantibodies. *Tissue Antigens*, 22, 2, (Aug, 1983) pp. 107-117
- Reichert, J. M. (2011). Antibody-based therapeutics to watch in 2011. *MAbs*, 3, 1, (Jan-Feb, 2011) pp. 76-99
- Reinhardt, C., *et al.* (2010). Results of a randomized Phase 2 study investigating multi-peptide vaccination with IMA901 in advanced renal cell carcinoma (RCC). *Proceedings of the American Society of Clinical Oncology (ASCO)*, Chicago, IL, USA, June 2010
- Rini, B. I. (2005). VEGF-targeted therapy in metastatic renal cell carcinoma. *Oncologist*, 10, 3, (Mar, 2005) pp. 191-197
- Rini, B. I. & M. B. Atkins (2009). Resistance to targeted therapy in renal-cell carcinoma. *Lancet Oncol*, 10, 10, (Oct, 2009) pp. 992-1000
- Rini, B. I., *et al.* (2006). Adoptive immunotherapy by allogeneic stem cell transplantation for metastatic renal cell carcinoma: a CALGB intergroup phase II study. *Biol Blood Marrow Transplant*, 12, 7, (Jul, 2006) pp. 778-785
- Rini, B. I., *et al.* (2011). IMA901 multi-peptide vaccine randomized INTernational phase III trial (IMPRINT): A randomized, controlled Phase III study investigating IMA901 multi-peptide cancer vaccine in patients receiving sunitinib as first-line therapy for advanced/metastatic RCC, Retrieved 12 Jul 2011, Available from <http://www.immatics.com/>
- Rodriguez, P. C., *et al.* (2009). Arginase I-producing myeloid-derived suppressor cells in renal cell carcinoma are a subpopulation of activated granulocytes. *Cancer Res*, 69, 4, (Feb 15, 2009) pp. 1553-1560
- Roepstorff, P. & J. Fohlman (1984). Letter to the editors. *Biological Mass Spectrometry*, 11, 11, 1984) pp. 601--601
- Romero, J. M., *et al.* (2006). Analysis of the expression of HLA class I, proinflammatory cytokines and chemokines in primary tumors from patients with localized and metastatic renal cell carcinoma. *Tissue Antigens*, 68, 4, (Oct, 2006) pp. 303-310
- Saenz-Lopez, P., *et al.* (2010). Higher HLA class I expression in renal cell carcinoma than in autologous normal tissue. *Tissue Antigens*, 75, 2, (Feb, 2010) pp. 110-118
- Sahin, U., *et al.* (1995). Human neoplasms elicit multiple specific immune responses in the autologous host. *Proc Natl Acad Sci U S A*, 92, 25, (Dec, 1995) pp. 11810--11813
- Scheffer, S. R., *et al.* (2003). Apoptotic, but not necrotic, tumor cell vaccines induce a potent immune response in vivo. *Int J Cancer*, 103, 2, (Jan 10, 2003) pp. 205-211
- Schirle, M., *et al.* (2000). Identification of tumor-associated MHC class I ligands by a novel T cell-independent approach. *Eur J Immunol*, 30, 8, (Aug, 2000) pp. 2216-2225

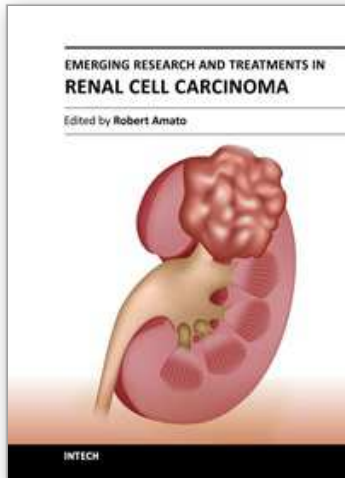
- Schulze, W. X. & B. Usadel (2010). Quantitation in mass-spectrometry-based proteomics. *Annu Rev Plant Biol*, 61, 2010) pp. 491-516
- Schwaab, T., et al. (2000). A randomized phase II trial comparing two different sequence combinations of autologous vaccine and human recombinant interferon gamma and human recombinant interferon alpha2B therapy in patients with metastatic renal cell carcinoma: clinical outcome and analysis of immunological parameters. *J Urol*, 163, 4, (Apr, 2000) pp. 1322-1327
- Schwaab, T., et al. (2009). Clinical and immunologic effects of intranodal autologous tumor lysate-dendritic cell vaccine with Aldesleukin (Interleukin 2) and IFN- α 2a therapy in metastatic renal cell carcinoma patients. *Clin Cancer Res*, 15, 15, (Aug 1, 2009) pp. 4986-4992
- Seliger, B., et al. (2002). HLA class I antigen abnormalities and immune escape by malignant cells. *Semin Cancer Biol*, 12, 1, (Feb, 2002) pp. 3-13
- Seliger, B., et al. (2003). Detection of renal cell carcinoma-associated markers via proteome- and other 'ome'-based analyses. *Brief Funct Genomic Proteomic*, 2, 3, (Oct, 2003) pp. 194-212
- Serafini, P., et al. (2004). High-dose granulocyte-macrophage colony-stimulating factor-producing vaccines impair the immune response through the recruitment of myeloid suppressor cells. *Cancer Res*, 64, 17, (Sep 1, 2004) pp. 6337-6343
- Serafini, P., et al. (2008). Myeloid-derived suppressor cells promote cross-tolerance in B-cell lymphoma by expanding regulatory T cells. *Cancer Res*, 68, 13, (Jul 1, 2008) pp. 5439-5449
- Shimizu, K., et al. (2001). Enhancement of tumor lysate- and peptide-pulsed dendritic cell-based vaccines by the addition of foreign helper protein. *Cancer Res*, 61, 6, (Mar 15, 2001) pp. 2618-2624
- Sica, G. L., et al. (2003). B7-H4, a molecule of the B7 family, negatively regulates T cell immunity. *Immunity*, 18, 6, (Jun, 2003) pp. 849-861
- Siebels, M., et al. (2011). A clinical phase I/II trial with the monoclonal antibody cG250 (RENCAREX(R)) and interferon-alpha-2a in metastatic renal cell carcinoma patients. *World J Urol*, 29, 1, (Feb, 2011) pp. 121-126
- Simons, J. W. & B. Mikhak (1998). Ex-vivo gene therapy using cytokine-transduced tumor vaccines: molecular and clinical pharmacology. *Semin Oncol*, 25, 6, (Dec, 1998) pp. 661-676
- Slingluff, C. L., Jr., et al. (2009). Effect of granulocyte/macrophage colony-stimulating factor on circulating CD8+ and CD4+ T-cell responses to a multipeptide melanoma vaccine: outcome of a multicenter randomized trial. *Clin Cancer Res*, 15, 22, (Nov 15, 2009) pp. 7036-7044
- Smigielski, E. M., et al. (2000). dbSNP: a database of single nucleotide polymorphisms. *Nucleic Acids Res*, 28, 1, (Jan, 2000) pp. 352-355
- Spits, H., et al. (1983). Characterization of monoclonal antibodies against cell surface molecules associated with cytotoxic activity of natural and activated killer cells and cloned CTL lines. *Hybridoma*, 2, 4, 1983) pp. 423-437
- Stevanovic, S. (2002). Identification of tumour-associated T-cell epitopes for vaccine development. *Nat Rev Cancer*, 2, 7, (Jul, 2002) pp. 514-520

- Stickel, J. S., *et al.* (2011). Quantification of HLA class I molecules on renal cell carcinoma using Edman degradation. *BMC Urol*, 11, pp. 1
- Stratton, M. R. (2011). Exploring the genomes of cancer cells: progress and promise. *Science*, 331, 6024, (Mar 25, 2011) pp. 1553-1558
- Sturm, M., *et al.* (2008). OpenMS - an open-source software framework for mass spectrometry. *BMC Bioinformatics*, 9, 2008) pp. 163
- Stutman, O. (1979). Spontaneous tumors in nude mice: effect of the viable yellow gene. *Exp Cell Biol*, 47, 2, 1979) pp. 129-135
- Suekane, S., *et al.* (2007). Phase I trial of personalized peptide vaccination for cytokine-refractory metastatic renal cell carcinoma patients. *Cancer Sci*, 98, 12, (Dec, 2007) pp. 1965-1968
- Sufan, R. I., *et al.* (2004). The role of von Hippel-Lindau tumor suppressor protein and hypoxia in renal clear cell carcinoma. *Am J Physiol Renal Physiol*, 287, 1, (Jul, 2004) pp. F1-6
- Tani, K., *et al.* (2004). Phase I study of autologous tumor vaccines transduced with the GM-CSF gene in four patients with stage IV renal cell cancer in Japan: clinical and immunological findings. *Mol Ther*, 10, 4, (Oct, 2004) pp. 799-816
- Tatsugami, K., *et al.* (2008). Dendritic cell therapy in combination with interferon-alpha for the treatment of metastatic renal cell carcinoma. *Int J Urol*, 15, 8, (Aug, 2008) pp. 694-698
- Taylor, A., *et al.* (2006). Mechanisms of immune suppression by interleukin-10 and transforming growth factor-beta: the role of T regulatory cells. *Immunology*, 117, 4, (Apr, 2006) pp. 433-442
- Therasse, P., *et al.* (2000). New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst*, 92, 3, (Feb 2, 2000) pp. 205-216
- Thompson, R. H., *et al.* (2005). Costimulatory molecule B7-H1 in primary and metastatic clear cell renal cell carcinoma. *Cancer*, 104, 10, (Nov 15, 2005) pp. 2084-2091
- Thornton, A. M. & E. M. Shevach (2000). Suppressor effector function of CD4+CD25+ immunoregulatory T cells is antigen nonspecific. *J Immunol*, 164, 1, (Jan 1, 2000) pp. 183-190
- Topalian, S. L., *et al.* (1988). Immunotherapy of patients with advanced cancer using tumor-infiltrating lymphocytes and recombinant interleukin-2: a pilot study. *J Clin Oncol*, 6, 5, (May, 1988) pp. 839-853
- Toussaint, N. C. & O. Kohlbacher (2009). OptiTope--a web server for the selection of an optimal set of peptides for epitope-based vaccines. *Nucleic Acids Res*, 37, Web Server issue, (Jul, 2009) pp. W617--W622
- Tureci, O., *et al.* (2005). Identification of tumor-associated autoantigens with SEREX. *Methods Mol Med*, 109, pp. 137-154
- Tykodi, S. S., *et al.* (2004). Allogeneic hematopoietic cell transplantation for metastatic renal cell carcinoma after nonmyeloablative conditioning: toxicity, clinical response, and immunological response to minor histocompatibility antigens. *Clin Cancer Res*, 10, 23, (Dec 1, 2004) pp. 7799-7811

- Uemura, H., *et al.* (2006). A phase I trial of vaccination of CA9-derived peptides for HLA-A24-positive patients with cytokine-refractory metastatic renal cell carcinoma. *Clin Cancer Res*, 12, 6, (Mar 15, 2006) pp. 1768-1775
- Uyttenhove, C., *et al.* (2003). Evidence for a tumoral immune resistance mechanism based on tryptophan degradation by indoleamine 2,3-dioxygenase. *Nat Med*, 9, 10, (Oct, 2003) pp. 1269-1274
- Van den Hove, L. E., *et al.* (1997). Phenotype, cytokine production and cytolytic capacity of fresh (uncultured) tumour-infiltrating T lymphocytes in human renal cell carcinoma. *Clin Exp Immunol*, 109, 3, (Sep, 1997) pp. 501-509
- Visvader, J. E. & G. J. Lindeman (2008). Cancer stem cells in solid tumours: accumulating evidence and unresolved questions. *Nat Rev Cancer*, 8, 10, (Oct, 2008) pp. 755-768
- von Boehmer, H. (2005). Mechanisms of suppression by suppressor T cells. *Nat Immunol*, 6, 4, (Apr, 2005) pp. 338-344
- Wang, H. Y., *et al.* (2004). Tumor-specific human CD4+ regulatory T cells and their ligands: implications for immunotherapy. *Immunity*, 20, 1, (Jan, 2004) pp. 107-118
- Wang, J. C. & J. E. Dick (2005). Cancer stem cells: lessons from leukemia. *Trends Cell Biol*, 15, 9, (Sep, 2005) pp. 494-501
- Wang, K., *et al.* (2009). Identification of tumor-associated antigens by using SEREX in hepatocellular carcinoma. *Cancer Lett*, 281, 2, (Aug 28, 2009) pp. 144-150
- Weber, F., *et al.* (2005). Variability in organ-specific EGFR mutational spectra in tumour epithelium and stroma may be the biological basis for differential responses to tyrosine kinase inhibitors. *Br J Cancer*, 92, 10, (May 23, 2005) pp. 1922-1926
- Wei, Y. C., *et al.* (2007). Combined treatment of dendritoma vaccine and low-dose interleukin-2 in stage IV renal cell carcinoma patients induced clinical response: A pilot study. *Oncol Rep*, 18, 3, (Sep, 2007) pp. 665-671
- Weinzierl, A. O., *et al.* (2007). Distorted relation between mRNA copy number and corresponding major histocompatibility complex ligand density on the cell surface. *Mol Cell Proteomics*, 6, 1, (Jan, 2007) pp. 102-113
- Welters, M. J., *et al.* (2009). Report on the sixth annual meeting of the Association for Immunotherapy of Cancer (CIMT), May 15 and 16, 2008 in Mainz, Germany. *Cancer Immunol Immunother*, 58, 5, (May, 2009) pp. 777-787
- Wierceky, J., *et al.* (2006). Immunologic and clinical responses after vaccinations with peptide-pulsed dendritic cells in metastatic renal cancer patients. *Cancer Res*, 66, 11, (Jun 1, 2006) pp. 5910-5918
- Wolchok, J. D., *et al.* (2009). Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. *Clin Cancer Res*, 15, 23, (Dec 1, 2009) pp. 7412-7420
- Wong, K. M., *et al.* (2011). Unraveling the Genetics of Cancer: Genome Sequencing and Beyond. *Annu Rev Genomics Hum Genet*, (Jun 2, 2011)
- Woo, E. Y., *et al.* (2001). Regulatory CD4(+)/CD25(+) T cells in tumors from patients with early-stage non-small cell lung cancer and late-stage ovarian cancer. *Cancer Res*, 61, 12, (Jun 15, 2001) pp. 4766-4772
- Wood, C., *et al.* (2008). An adjuvant autologous therapeutic vaccine (HSPPC-96; vitespen) versus observation alone for patients at high risk of recurrence after nephrectomy

- for renal cell carcinoma: a multicentre, open-label, randomised phase III trial. *Lancet*, 372, 9633, (Jul 12, 2008) pp. 145-154
- World Health Organization., (1979). *WHO handbook for reporting results of cancer treatment*, World Health Organization, ISBN: 9241700483, Geneva, Albany, N.Y.
- Yates, J. R., *et al.* (2009). Proteomics by mass spectrometry: approaches, advances, and applications. *Annu Rev Biomed Eng*, 11, (2009) pp. 49-79
- Young, M. R., *et al.* (1997). Increased recurrence and metastasis in patients whose primary head and neck squamous cell carcinomas secreted granulocyte-macrophage colony-stimulating factor and contained CD34+ natural suppressor cells. *Int J Cancer*, 74, 1, (Feb 20, 1997) pp. 69-74
- Zea, A. H., *et al.* (2005). Arginase-producing myeloid suppressor cells in renal cell carcinoma patients: a mechanism of tumor evasion. *Cancer Res*, 65, 8, (Apr 15, 2005) pp. 3044-3048
- Zou, W. (2006). Regulatory T cells, tumour immunity and immunotherapy. *Nat Rev Immunol*, 6, 4, (Apr, 2006) pp. 295-307

IntechOpen



Emerging Research and Treatments in Renal Cell Carcinoma

Edited by Dr. Robert Amato

ISBN 978-953-51-0022-5

Hard cover, 442 pages

Publisher InTech

Published online 03, February, 2012

Published in print edition February, 2012

The field of renal cell cancer has undergone a significant resurgence. This book summarizes up-to-date research and innovative ideas for the future in this rapidly changing field, which encompasses medicine, surgery, radiation oncology, basic science, pathology, radiology, and supportive care. This book is aimed at the clinician or scientist who has an interest in renal cell cancer, whether they are academic or nonacademic. The book covers tumor biology, molecular biology, surgery techniques, radiation therapy, personal testimonies, and present and future treatments of the disease that are on the horizon. The goal was to produce a textbook that would act as an authoritative source for scientists and clinicians and interpret the field for trainees in surgery, medicine, radiation oncology, and pathology.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Heiko Schuster, Mathias Walzer and Stefan Stevanović (2012). Immunotherapy of Renal Cell Carcinoma – From Antigen Identification to Patient Treatment, *Emerging Research and Treatments in Renal Cell Carcinoma*, Dr. Robert Amato (Ed.), ISBN: 978-953-51-0022-5, InTech, Available from: <http://www.intechopen.com/books/emerging-research-and-treatments-in-renal-cell-carcinoma/immunotherapy-of-renal-cell-carcinoma-from-antigen-identification-to-patient-treatment>

INTECH
open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen