Chapter 5

Autologous Immunotherapy as a Novel Treatment for Bladder Cancer

Martin C. Schumacher and Amir M. Sherif

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1. Introduction

Cancer is fundamentally a disease with failure in regulation in tissue growth and the risk of developing cancer increases with age. The armamentarium in treating cancer is mainly threefold: surgical resection of the tumor, radiation therapy and cytotoxic drugs. For bladder cancer, results from contemporary radical cystectomy series with pelvic lymph node dissection for T2-4 NX M0 transitional cell carcinoma (TCC) provides accurate pathologic staging of the primary tumor and lymph nodes, and due to increasing expertise with the different types of urinary diversions durable preservation of quality of life. However, the 5-year survival rate for all patients with pT2 tumors is approximately 50 – 80%, and for those with negative lymph nodes 64 – 86% (Stein, Lieskovsky et al. 2001) (Shariat, Karakiewicz et al. 2006) (Hautmann, de Petriconi et al.). In contrast, the 5-year survival rates for locally advanced cancers, pT3 and pT4, in contemporary cystectomy series range from 22 – 58%. The presence of pathologically proven lymph node metastases at radical cystectomy is associated with a poor outcome with a 5-year survival of 30%.

After more than 30 years of clinically research in bladder cancer, the true role of neoadjuvant and adjuvant chemotherapy for locally advanced bladder cancer remains unclear. Neoadjuvant chemotherapy has been shown to help for debulking and facilitation for surgical resection at radical cystectomy. The overall survival benefit is unfortunately relatively low (< 9%) and treatment protocols are often not suitable in older patients with comorbidities and decreased renal function (Grossman, Natale et al. 2003) (Sonpavde, Amiel et al. 2008) (2005). Identification of responders versus non-responders to neoadjuvant chemotherapy seems to be of value for selection of patients to be treated with this modality, still at present - robust and readily available markers predicting treatment response are lacking (Rosenblatt, Sherif et al.). Adjuvant chemotherapy trials have been

less clear, with at least a trend of improved disease-free survival in small series of statistically underpowered trials (Walz, Shariat et al. 2008). Thus, other treatment modalities are highly warranted for these patients.

Immunotherapy offers an appealing complement to traditional chemotherapy, with possible long-term protection against tumor recurrences through immunological memory. Vaccination trials have shown promising results in colorectal cancer patients (Hanna, Hoover et al. 2001) (Mocellin, Rossi et al. 2004) (Karlsson, Marits et al.). Similar studies have been performed in patients with malignant melanoma (Dudley, Wunderlich et al. 2005). Adoptive immunotherapy with the collection and expansion of autologous tumor-reactive lymphocytes, followed by re-transfusion to the patient, has been reported to influence tumor progression. Another approach, using a combination between adoptive immunotherapy and a retroviral gene therapy using specific malignant melanoma T cell receptors, showed sustained levels of circulating, engineered cells at one year after infusion in two patients who both demonstrated objective regression of metastatic melanoma lesions (Morgan, Dudley et al. 2006).

Due to promising results using adoptive immunotherapy, our interests turned to bladder cancer, as few new cytotoxic drugs are available. This review provides an overview on the concept of sentinel node detection, necessary for the collection and expansion of autologous tumor-reactive lymphocytes in bladder cancer patients, as a novel adoptive immunotherapy.

2. Immunotherapy as cancer therapy

Over the past decade, interest has turned to other treatment concepts as novel cancer strategies than cytotoxic drugs. A variety of immunotherapeutic approaches have been tested in order to stimulate the cellular and humoral arms of the immune system to induce tumor regression. Currently, the following treatment strategies seem most promising, including the application of cytokines and adjuvant agents which modulate the cytokine response, cancer vaccines designed to elicit cellular immune responses against tumor associated antigens (TAAs), and monoclonal antibody drugs (Kusmartsev and Vieweg 2009).

Despite better understanding of the immune system only a few immunotherapeutic approaches have received approval by the Federal Drug Agency (FDA) for treatment of urological malignancies, such as the systemic administration of interleukin (IL-2) against metastatic renal cell cancer (RCC) and the intravesical instillation of bacillus Calmette-Guérin (BCG) or interferon α for non-muscle-invasive bladder cancer. The cancer vaccine that has received the most publicity and attention is undoubtedly Sipuleucel-T or Provenge® (Lubaroff 2012). The vaccine was approved by the FDA in April 2010 for men with asymptomatic or minimal symptomatic castration resistant prostate cancer (CRPC).

Cancer vaccines are designed to stimulate expansion of the cellular arm of the immune system, mainly T cells and natural killer cells. Cytotoxic and helper T lymphocytes are consid-

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ered the main immune effector cells, which in turn kill tumor cells via receptor mediated interactions. Both cell types require activation by antigen presenting cells, such as dendritic cells (DCs), to recognize and kill tumor cells in context with major histocompatibility complex (MHC) self-antigens. Natural killer cells, by contrast, kill rather non-specifically and represent the first line of immunological defense against cancer and foreign pathogens. Many vaccine approaches have shown high efficacy at triggering T-cell responses against TAAs in tumor bearing animals—these approaches include vaccination with gene modified tumor cells, antigen-loaded DCs, recombinant viral expression cassettes, and heat shock proteins (Kusmartsev and Vieweg 2009).

Despite the fact that many immunologic approaches have moved from basic research into clinical trials, only a few showed clinical response and tumor regression. As the rates of tumor regression has seldom exceeded 5 - 10%, with only a short duration of clinical response, the efficacy of these treatments has been seriously questioned (Vieweg and Dannull 2005). A possible explanation for the limited response of cancer vaccines lies in the fact that new drugs must be initially studied in patients with advanced or metastatic disease, with poor survival outcome. Additionally, the immunogenicity of the TAAs used in reported vaccine formulations is low, as most TAAs represent self-antigens that are overexpressed or reactivated in cancer cells relative to the non-cancerous cells from which they originated. Finally, tumors can evade the immune system (including the immune responses triggered by vaccination) through the induction of immune tolerance or immune suppression (Kusmartsev and Vieweg 2009) (Gilboa 2004) (Rabinovich, Gabrilovich et al. 2007).

In times of economic uncertainties, cancer vaccine treatments are not without controversy. The controversial issues that have been raised in using Sipuleucel-T include the high cost, the modest improvement in overall survival (OS) and virtually an absence of change in time to progression (Chambers and Neumann 2011) (Goozner 2011). Priced at $31,000 per treatment, with a usual course of three treatments, Sipuleucel-T is one of the most expensive cancer therapies ever to hit the marketplace. Whether, health care providers can afford these additional burden remains to be seen in the near future.

3. Immunotherapy in non-advanced urothelial carcinoma

Bladder cancer is the fifth most commonly diagnosed cancer in the US in 2012 (after prostate, breast, lung, and colon cancers), with an estimated 73’510 new cases and 14’880 deaths (2012). Risk factors for developing bladder cancer include cigarette smoking, exposure to arsenic, occupation in rubber or fossil oil industry, and schistosomiasis, and chronic inflammatory disease (Steineck, Plato et al. 1990). Approximately 70% to 80% of patients with newly diagnosed bladder cancer will present as noninvasive papillary transitional-cell carcinomas (TCCs), 70% of which will recur, and 10 – 20% of which will progress and invade the bladder wall (Babjuk, Oosterlinck et al. 2012). Those who do present with superficial, noninvasive bladder cancer can often be cured, and those with

deeply invasive disease can sometimes be cured by surgery, radiation therapy, or a combination of modalities that include chemotherapy.

According to the National Cancer Institute's Surveillance Epidemiology and End Results (SEER) registry, there has been a gradually increasing incidence of bladder cancer over the past three decades (Kamel, Moore et al.). The incidence of muscle invasive tumors has remained stable over this time; however, the incidence of superficial, noninvasive bladder cancer is rising.

Transurethral resection of bladder tumor (TURBT) is the standard initial therapeutic approach for diagnosis and treatment of nonmuscle invasive bladder cancer (NMIBC) (Babjuk, Oosterlinck et al. 2012) (Williams, Hoenig et al. 2010) (Brausi, Witjes et al.). However, although TURBT is an effective therapy, up to 45% of patients will experience tumor recurrence within 1 year after TURBT alone (Hall, Chang et al. 2007). Additionally, a 3 to 15% risk of tumor progression to muscle-invasive and/or metastatic cancer has been reported. According to these figures TURBT alone is considered to be an insufficient treatment modality in most patients. To overcome the limitations of TURBT alone, interest has turned towards adjuvant intravesical treatment regimens since the early 1970s.

The difficulty in the management of bladder cancer comes from the inability to predict which tumors will recur or progress. Current evidence suggest for the existence of mutually exclusive molecular pathways to tumorigenesis, responsible for the formation of papillary and invasive carcinomas, respectively (Wolff, Liang et al. 2005). The most common genetic alterations in low grade papillary TCC are loss of heterozygosity of part or all of chromosome 9 and activating mutations of the fibroblast growth factor receptor 3 (*FGFR3*) (Cappellen, De Oliveira et al. 1999; Cheng, Huang et al. 2002; Bakkar, Wallerand et al. 2003). In contrast to the pathway responsible for the development of invasive TCC which seems to start with dysplasia, progress to carcinoma *in situ*, followed by invasion of the lamina propria. The most frequent genetic alteration in dysplasia and carcinoma *in situ* is mutation of *TP53,* followed by loss of heterozygosity of chromosome 9 (Burkhard, Markwalder et al. 1997; Orlow, LaRue et al. 1999; Sarkar, Julicher et al. 2000; Hartmann, Schlake et al. 2002). An important marker for progression in TCC is loss of chromosome 8p, which occurs in approximately 60% of bladder tumors (Stoehr, Wissmann et al. 2004). Global trends of increased genomic instability and aberrant methylation of cytosine residues in DNA correlate with increased tumor invasion and progression (Dulaimi, Uzzo et al. 2004). This may partly explain why the incidence of superficial, noninvasive bladder cancer is rising (Kamel, Moore et al.).

4. Intravesical immunotherapy

Bacillus Calmette-Guerin (BCG) is the most commonly used first-line immunotherapeutic agent for prophylaxis and treatment of carcinoma in situ and high-grade bladder cancer. BCG has fundamentally changed the management of high risk nonmuscle invasive TCC, particularly carcinoma in situ (CIS) (Babjuk, Oosterlinck et al.). The aim of adjuvant intra-

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vesical immunotherapy is to avoid post-TURBT implantation of tumor cells, eradicate residual cancer cells and delay tumor recurrence by local immunostimulation (Soloway, Nissenkorn et al. 1983). The effect on cancer progression is unclear.

Other immunotherapeutic drugs include the interferons (IFN), interleukin (IL-2, IL-12), as well as tumor necrosis factor (TNF), which have their place in BCG-refractory patients (Glazier, Bahnson et al. 1995; Magno, Melloni et al. 2002; Weiss, O'Donnell et al. 2003).

5. Bacillus Calmette-Guerin (BCG)

BCG is a live-attenuated vaccine, and until today considered to be the most effective intravesical treatment for carcinoma in situ and high grade stage Ta or T1 TCC (Shelley, Kynaston et al. 2001; Han and Pan 2006). It was developed by Albert Calmette and Camille Guerin in 1921 at the Pasteur Institute in France by attenuating the bovine tuberculous bacillus, Mycobacterium bovis (Calmette 1931; Herr and Morales 2008). The background of the antitumor properties of BCG is based on observational autopsy studies in tuberculosis patients which had a lower frequency of various tumors (Pearl 1929). Old et al. were the first to demonstrate a potential benefit using BCG in infected mice who showed increased resistance to challenge with transplantable tumors (Old, Clarke et al. 1959). Ten years later Mathe et al. reported encouraging results with BCG as adjuvant therapy for acute lymphoblastic leukemia (Mathe, Pouillart et al. 1969). In 1976, Morales et al. were the first to report the successful use of BCG in the treatment of bladder cancer (Morales, Eidinger et al. 1976).

The exact mechanisms of action and its antitumor properties of BCG in bladder cancer remains to be elucidated. However, immediately after intravesical instillation, BCG infects and is internalized into urothelial and bladder cancer cells via a fibronectin-dependent process mediated by integrins (Becich, Carroll et al. 1991; Kuroda, Brown et al. 1993). Fibronectin attachment protein (FAP) mediates BCG attachment to bladder cancer cells and the urothelial wall following intravesical instillation. The interaction between BCG with urothelial cells results in several immunologically changes, including induction of chemokines such as interleukin (IL)-1, IL-6, IL-8, IL-17 [18], GM-CSF, tumor necrosis factor (TNF), and the up-regulation of intracellular adhesion molecule (ICAM)-1 (Alexandroff, Jackson et al. 1999; Simons, O'Donnell et al. 2008). These cytokines are considered critical for cellular assault by causing tumor cells to display molecules that serve as attachment anchors for immune cells, including neutrophils and T lymphocytes, and activation signals such as ICAM-1, fatty-acid synthetase (FAS), CD40, *etc* (Alexandroff, Jackson et al. 1999; Wolff, Liang et al. 2005). The importance of these immunologic changes can be partly assessed by the high level of IL-8 production which is associated with better clinical responses to BCG (Thalmann, Dewald et al. 1997; Thalmann, Sermier et al. 2000).

After weekly intravesical instillations of BCG, a variety of immune cells such as neutrophils, macrophages, natural killer cells, T lymphocytes, and NKT cells are constantly

recruited. Urinary samples from patients under BCG instillation therapy contain almost seventy-five percent of neutrophils, five to ten percent of macrophages and one to three percent of NK cells (De Boer, De Jong et al. 1991). The neutrophils secrete cytokines which in turn activate various effector cells. To achieve an immunologic reaction and a potential therapeutic effect it takes five to six BCG instillations (Prescott, James et al. 1992) (Jackson, Alexandroff et al. 1995).

Potential effector cells responsible for tumor killing include MHC-nonrestricted cells such as NK cells lymphokine-activated killer (LAK) cells, BCG-activated killer cells, CD-1-restricted CD8+ T cells, gd T cells, NKT cells, neutrophils, macrophages, and MHC-restricted CD8+ and CD4+ T cells (Kitamura and Tsukamoto 2011). Of these cells, T lymphocytes are considered to be the most effective effector cells responsible for eliminating cancer cells (Alexandroff, Nicholson et al.). In a depletion study, both CD8+ and CD4+ T cells were found to be essential for the successful antitumor effects of BCG (Ratliff, Ritchey et al. 1993).

According to the current literature at least four meta-analyses have shown that TURBT plus intravesical BCG is superior to TURBT alone for delaying time to first tumor recurrence (Shelley, Kynaston et al. 2001; Bohle and Bock 2004; Shelley, Wilt et al. 2004; Han and Pan 2006). The largest meta-analysis by the EORTC reviewed data from 24 randomized trials and reported that the progression rate in the group TURBT plus BCG was 9.8% vs. 13.8% in the control groups with a median follow-up of 2.5 years (maximum up to 15 years) (Pawinski, Sylvester et al. 1996). Despite the fact that BCG may delay tumor progression, patients are still at risk for metastatic or muscle-invasive disease. This has been highlighted in the study by Lamm et al. on the natural history of untreated CIS with a progression rate to muscle-invasive disease in 54% (Lamm 1992).

Even with initial complete response after BCG treatment regimens, there is a continued risk for tumor recurrence or the occurrence of new tumors on the long-term. Thus, according to the risk assessment of the tumor lifelong surveillance is mandatory.

The administration of intravesical BCG, as well as its optimum dose and treatment schedule remains under investigation. Until today the original treatment protocol by Morales et al. of six instillations, once a week for six weeks, is still considered standard of care (Morales, Eidinger et al. 1976). Cystoscopy with urinary cytology is performed six weeks after completion of BCG instillation to assess treatment response (Babjuk, Oosterlinck et al.).

6. Interferons (INFs)

IFNs are host-produced glycoproteins which act to mediate immune responses through antiviral, antiproliferative, and immuneregulatory activities (Williams, Hoenig et al.). In vitro studies have shown that INFs have direct antitumor effects (Baron, Tyring et al. 1991). INF-α2b has been the most extensively studied interferon as an intravesical agent, and it seems that the in vitro effect of antiproliferative activity on bladder cancer cells are also observed in vivo (Molto, Alvarez-Mon et al. 1995). Several studies comparing the antitumor activity of INF-α2b com-

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pared with BCG, demonstrated a clear inferiority regarding risk for recurrence or time to first recurrence (Kalble, Beer et al. 1994; Portillo, Martin et al. 1997). For this reason, and the high costs of INF-α2b, INF-α2b has been mainly used for salvage treatment protocols, as BCG failure patients have a 15 - 20% complete response to INF-α2b at one year (Lam, Benson et al. 2003).

In order to determine whether mitomycin C followed by BCG vs. BCG plus IFN-a2b decreased the intravesical recurrence rate, a randomized study could demonstrate that there is no benefit by alternating IFN-a2b with BCG (Kaasinen, Rintala et al. 2000). Thus addition of IFN-a to BCG does not seem to enhance the antitumor effects of BCG immunotherapy.

7. Future perspectives for intravesical treatments

Besides urgent need for tumor markers in bladder cancer patients to better detect recurrences, attempts are under investigation for optimal drug delivery using intravesical treatments. Different devices are currently under investigation such as thermochemotherapy and electromotive drug administration in non-muscle invasive bladder tumors. The idea behind these drug delivery approaches is to temporarily breach the urothelium which in turn should lead in an increased accumulation of drugs in the bladder tissue. First results are encouraging using electromotive mitomycin C (eMMC) instillations in patients with CIS, with a statistically significant, superior complete response rate at 6 months for eMMC (58%) compared to passive MMC at the higher doses (31%) (Di Stasi, Giannantoni et al. 2003). The response rate of eMMC approached that of BCG (64%). Local microwave hyperthermia (Synergo system) is another technology being investigated in the treatment of bladder cancer. The Synergo system stimulates bladder wall hyperthermia through an energy delivering unit in the tip of a special catheter equipped with internal thermocouples designed to maintain temperatures between 42 and 43°C. The aim is to increases cell-membrane permeability and by this way alter intracellular drug trafficking and distribution (Moskovitz, Meyer et al. 2005). Whether these combined approaches using thermal energy and intravesical agents will revolutionize the treatment of bladder cancer remains to be seen in the future (Williams, Hoenig et al. 2010).

Another approach has been reported by Sharma et al. in a post TURBT adjuvant setting (Sharma, Bajorin et al. 2008). The safety and immunogenicity of a recombinant NY-ESO-1 protein vaccine, which was administered with granulocyte macrophage colony-stimulating factor and BCG as immunologic adjuvant was tested in a cohort of urothelial carcinoma patients. Six patients met all eligibility criteria to receive the vaccination after TURBT for localized TCC. Tumor tissues were tested for NY-ESO-1 expression and patients, shown to have NY-ESO-1 tumors, were vaccinated in the postoperative setting. Peripheral blood samples were analyzed for vaccine-induced antibody and T-cell responses. NY-ESO-1-specific antibody responses were induced in 5/6 patients whereas CD8 T-cell responses occurred in 1/6 patients and CD4 T-cell responses were found in 6/6 patients. This study demonstrates safety and feasibility of the NY-ESO-1 recombinant protein in combination with BCG and granulocyte macrophage colony-stimulating factor to induce predominantly antibody and CD4 T-cell responses in urothelial carcinoma patients. Induction of higher frequency of CD8 T-

cell responses may be possible in clinical trials implementing NY-ESO-1 vaccination in combination with other immunomodulatory agents (Sharma, Bajorin et al. 2008).

8. Sentinel lymph node concept, detection and clinical implications

The sentinel node (SN) is defined as the first tumor-draining lymph node along the direct drainage route from the tumor; in case of dissemination, it is considered being the first site of metastasis. A tumor can have more than one primary sentinel node, due to different sections of the tumor being drained. In a defined micro-anatomical drainage route, the first node is called the first echelon SN followed by the second echelon SN, the third and so forth (figure 1). Identification and subsequent pathologic examination of the SNs reflects the nodal status of the remaining regional nodes. It is postulated that regional nodes in the vicinity, which are unconnected to the tumor draining routes, by definition cannot be or become hosts of tumor dissemination. The concept of a sentinel node was first described 1960, in a patient with cancer of the parotid gland (Gould, Winship et al. 1960). Detection of the SN was further introduced in urology by Cabanas in 1977, aiming at improved accuracy in penile carcinoma staging (Cabanas 1977). The SN technique is now established as a routine method in malignant melanoma and breast cancer. SN detection is still experimental in urologic malignancies and is previously described in urinary bladder cancer (Sherif, De La Torre et al. 2001) (Sherif, Garske et al. 2006) (Liedberg, Chebil et al. 2006), in prostate cancer (Wawroschek, Vogt et al. 1999) (Jeschke, Nambirajan et al. 2005), in testicular cancer (Ohyama, Chiba et al. 2002) and in renal cell carcinoma (Sherif, Eriksson et al.) (Bex, Vermeeren et al.). A further extension of the concept is in identification of Metinel nodes (MN), which are defined as lymph nodes draining a metastatic site (Dahl, Karlsson et al. 2008). This might have further implications in subsequent immunological therapies based on using tumor extract as antigen source, due to the presence of intratumor heterogeneity both in primary tumors (Gerlinger, Rowan et al.) and the suggested clonal differentiation displayed in metastatic sites (Malmstrom, Ren et al. 2002).

Various procedures entailing/techniques for sentinel node detection:

- Preoperative planar lymphoscintigraphy
- Preoperative planar lymphoscintigraphy in conjunction with SPECT/CT [single photone-mission CT (SPECT) with a low-dose CT]
- Intraoperative visual blue dye detection
- Intraoperative gamma probe/Geiger meter-detection
- Postoperative scintigraphy of main specimen with planar acquisition

In most centers one, two or three methods combined are considered being sufficient for the everyday clinical praxis.

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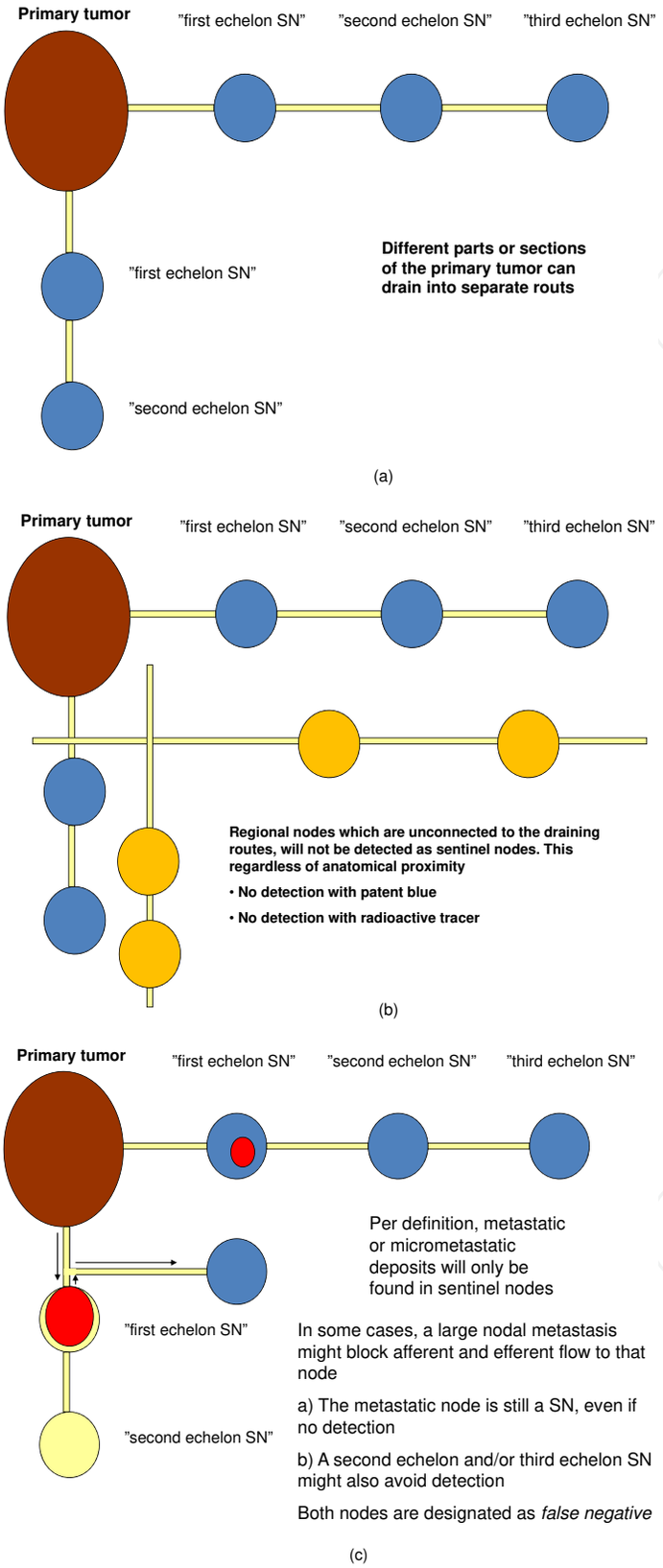

Figure 1.

9. T-cell function, including receptor and antigen recognition

The subset of lymphocytes called T cells mature in the thymus and are distinguished from other lymphocytes (B-cells, NK-cells) by their T cell receptor (TCR) located on the cell surface. Different subsets of T cells display a variety of different functions:

- T helper cell (T_H cells) [also known as CD4 cells]
- Cytotoxic T cells (CTLs or T_C) [also known as CD8 cells]
- Memory T cells
  - Central memory T cells (T_{CM} cells)
  - Effector memory T cells (T_{EM} cells)
- Regulatory T cells (T_{reg} cells)
  - Naturally occurring T_{reg} cells
  - Adaptive T_{reg} cells
- Natural killer T cells (NKT cells)
- γδ T cells (gamma delta T cells)

The origin of all T cells is the hematopoietic stem cells in the bone marrow. Immature thymocytes do not express any of the two markers CD4 or CD 8. During the development of the thymocytes, they finally become either of the two major subsets followed by release to peripheral tissues. Prior to the release, the TCR has developed on the surface through different selection processes in the thymus, enabling the future mature T cell to interact with MHC/HLA complexes and also to have attained a balanced reaction to self-antigens. The T cells which exit the thymus are designated as *mature naive T cells*. The TCR is composed of two separate peptide chains joined in a complex with CD3-proteins. When the TCR is activated a number of processes take place finalizing in activation of the transcription factor NFAT (Nuclear factor of activated T-cells). NFAT translocates to the nucleus of the T cell and activates a number of genes as for instance IL-2, leading to growth, proliferation, and differentiation of the T cell. The TCR requests co-stimulation of CD28 also expressed on the T cell, for activation. In absence of interaction with CD28 when the T cell encounters APCs (antigen presenting cells), the T cell will not proliferate and the end result will be anergy and a suboptimal immunoresponse.

10. T-cell activation in lymph nodes

Animal models indicate that tumor antigensensitization of lymphocytes takes place in tumor draining lymph nodes (SNs and MNs), where tumor antigens are presented to T cells by specialized APCs (Itano and Jenkins 2003). Naive T lymphocytes are activated through

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their TCRs by peptide–MHC complexes displayed on dendritic cells in secondary lymphoid tissue (Jenkins, Khoruts et al. 2001). Upon activation, T cells undergo rapid proliferation, differentiating into effectors capable of migrating into various sites and of producing lymphokines. A contraction phase then results in the elimination of the vast majority of T cells, leaving behind a stable population of memory cells (Seder and Ahmed 2003).

11. Sentinel lymph node concept and immunology

In the sentinel nodes or metinel nodes, the antigen-presenting cells (most often dendritic cells) encounter tumor antigen, which is digested to peptides. The peptides are directed to the class 2 pocket and displayed on the cell surface for recognition by CD4+ T cells. Newly arrived T cells are guided to the T-cell zones of the node mainly by the chemokine CCL 21 through binding of the receptor CCR7 on the lymphocytes (Campbell, Bowman et al. 1998). On encountering the APCs, the naive T cells are specifically activated and undergo a clonal expansion.

Whereas effector memory cells are capable of executing immediate effector functions upon antigen encounter, central memory cells home to lymph nodes, may provide a lifelong source of new effector cells, both upon secondary stimulation and under the influence of homeostatic cytokines (Geginat, Sallusto et al. 2001) (Hammarlund, Lewis et al. 2003).

The tumor has its own line of defence when encountering an immunological assault in which is known as *tumor escape mechanisms*; thus tumor cells may escape elimination by losing targeted antigens, rendering T-cells anergic by downregulation of costimulatory molecules, by inducing regulatory T-lymphocytes (T-regs), or by specifically deleting responding T-lymphocytes (Staveley-O'Carroll, Sotomayor et al. 1998) (Woo, Yeh et al. 2002) (Engelhard, Bullock et al. 2002) (Lee, Yee et al. 1999).

12. Adoptive immunotherapy using autologous T-cells in bladder cancer: Results from the Karolinska University Hospital

Until now, only two pilot projects in humans describing immunotherapy using autologous T-cells collected from tumor draining lymph nodes followed by cell culture and expansion, have been published. The first one in advanced colon cancer and the second one in advanced urothelial bladder cancer. In 2006 our group described the possibility and the techniques of identifying, harvesting, enhancing, refining and multiplying mainly T helper cells (CD4+ Th1-lymphocytes) from draining sentinel lymph nodes in both colon cancer (Marits, Karlsson et al. 2006) and in bladder cancer (Marits, Karlsson et al. 2006). From there, the next step was taken and a treatment series of 16 patients with advanced colon cancer included between 2003-2008, were described (Karlsson, Nilsson et al. 2008). The selected patients were histopathologically classified as stage II, III or IV (AJCC criteria) tumors. The patients were followed for 36 months on average (range 6–51 months) and monitored in accordance

with the Swedish colorectal cancer follow-up protocol. The patients with distant metastases (stage IV) responded to treatment, either with extended periods of stable disease (n = 4), partial response with diminished tumor burden (n = 1) or complete response with no detectable remaining tumor (n = 4). The cumulative survival of the nine treated stage IV patients was compared with all stage IV cases in the Stockholm region during the year of 2003. The median survival of stage IV patients receiving immunotherapy was 2.6 years compared with 0.8 years median survival of the control group.

The same approach was used in urinary bladder cancer patients and the techniques and methods were published 2010 in the first 12 patients in an ongoing pilot trial (Sherif, Hasan et al. 2010). The preliminary results have so far included a total of 18 patients, in which 9 patients received intended treatment. Two of the nine treated patients showed objective responses by RECIST criteria, and also exceptionally long overall survival (Sherif et al 2011). Further evaluation and long-term follow-up results are necessary to assess the role of immunotherapy in bladder cancer patients.

13. Future perspectives

Recent research has suggested that chemotherapy in the traditional form not only exerts its effect on different moments in the cell cycle further leading to apoptosis, but also primarily and secondarily plays a major role in tumor immunological events (Demaria, Volm et al. 2001) (Hong, Puaux et al.) (Ramakrishnan, Huang et al.). A challenging option would be to combine neoadjuvant chemotherapy in high risk groups (non-responders and partial responders to cisplatine combination therapies) with adjuvant immunotherapy in one form or another. Hypothetically, neoadjuvant chemotherapy in urinary bladder cancer could be followed by sentinel node detection in conjunction with intended cystectomy. Primarily non-responders (>pT0) could be offered inclusion in a trial entailing treatment with autologous tumor-reactive lymphocytes.

14. Summary

According to the growing body of evidence in the understanding of molecular pathways in tumor biology, other treatment modalities than surgery, chemotherapy and radiotherapy will certainly increase our possibilities to treat various cancers. Immunotherapy provides the most exciting aspect for clinical research in the near future. As these treatments are mainly applied to patients with advanced diseases it remains to be seen whether early treatment strategy immunotherapy protocols will change the course of many diseases in the near future. To date, however, there have been only a few published phase I or II clinical trials of active immunotherapy for bladder cancer (table 1) (Sharma, Bajorin et al. 2008) (Honma, Kitamura et al. 2009) (Sherif, Hasan et al. 2010) (Malmstrom, Loskog et al.) (Matsumoto, Noguchi et al.).

Author	Treatment protocol	Disease stage	Number of patients	Phase study	Results	Side effects
Sharma et al. [2008]	NY-ESO-1 protein vaccine + CM-CSF + BCG	Adjuvant treatment post-TURBT	6	I	Ag-specific antibodies in 5/6 pts., CD8 T cell response in 1/6 Pts, CD4 T cell response in 6/6 pts.	Only mild injection site reactions
Honma et al. [2009]	Survivin-2B80-88 peptide vaccination	Advanced TCC	9	I	CD8 T cell response in 5/9 pts., tumor reduction in 1/9 pts.	No side effects
Sherif et al. [2010]	Reinfusion of autologous T-helper cells	T2-T4 N1-2 M0-1 bladder cancer	12	I	Feasible in 6/12 Pts, technical failure in 6/12 Pts,	No severe adverse events
Malmström et al. [2010]	Adenoviral vector expressing CD40 ligand (intravesical)	Muscle-invasive TCC scheduled for cystectomy (phase I), Ta disease (phase II)	8	I/II	Enhancement of T cell infiltration and IFN-γ production, reduction of circulating regulatory T-cells	No severe adverse events, minor local pain
Matsumoto et al. [2011]	Personalized peptide vaccine	Advanced TCC (MVAC failure)	10	I	1 CR, 1 PR, 2 SD, PFS 3.0 months, OS 8.9 months	No severe adverse events

MVAC: methotrexate,vinblastine, adriamycin and cisplatin; CR: complete response; PR: partial response; SD: stable disease, PFS: progression-free survival; OS: overall survival

Table 1. Present phase I and II clinical trials of active immunotherapy in bladder cancer

Author details

Martin C. Schumacher[1,2] and Amir M. Sherif[1]

1 Karolinska University Hospital, Dept. of Urology, Stockholm, Sweden

2 Hirslanden Klinik Aarau, Urology, Aarau, Switzerland

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