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REVIEW

Combination of active specific immunotherapy or adoptive antibody or lymphocyte immunotherapy with chemotherapy in the treatment of cancer

Tianqian Zhang · Dorothee Herlyn

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Abstract Successful treatment of cancer patients with a combination of monoclonal antibodies (mAb) and chemotherapeutic drugs has spawned various other forms of additional combination therapies, including vaccines or adoptive lymphocyte transfer combined with chemotherapeutics. These therapies were effective against established tumors in animal models and showed promising results in initial clinical trials in cancer patients, awaiting testing in larger randomized controlled studies. Although combination between immunotherapy and chemotherapy has long been viewed as incompatible as chemotherapy, especially in high doses meant to increase anti-tumor efficacy, has induced immunosuppression, various mechanisms may explain the reported synergistic effects of the two types of therapies. Thus direct effects of chemotherapy on tumor or host environment, such as induction of tumor cell death, elimination of regulatory T cells, and/or enhancement of tumor cell sensitivity to lysis by CTL may account for enhancement of immunotherapy by chemotherapy. Furthermore, induction of lymphopenia by chemotherapy has increased the efficacy of adoptive lymphocyte transfer in cancer patients. On the other hand, immunotherapy may directly modulate the tumor's sensitivity to chemotherapy. Thus, anti-tumor mAb can increase the sensitivity of tumor cells to chemotherapeutic drugs and patients treated first with immunotherapy followed by chemotherapy showed higher clinical response rates than patients that had received chemotherapy alone. In conclusion, combination of active specific immunotherapy or adoptive mAb or lymphocyte immunotherapy with chemotherapy has great potential for the treatment of cancer patients which needs to be confirmed in larger controlled and randomized Phase III trials.

Keywords Cancer · Immunotherapy · Chemotherapy · Antibody · Vaccine · Lymphocyte

Abbreviations

5-FC	5-Fluorocytosine
5-FU	5-Fluorouracil
Ab	Antibody
Ad	Adenovirus
Ag	Antigen
ADCC	Antibody-dependent cell-mediated cytotoxicity
AML	Acute myelogenous leukemia
APC	Antigen-presenting cells
BCG	Bacillus Calmette Guerin
BCNU	1, 3-Bis-(2-chloroethyl)-1-nitrosourea
CDC	Complement-dependent cytotoxicity
CDDP	Cisplatin
CEA	Carcinoembryonic antigen
CFA	Complete Freund's adjuvant
cFGFR	Chicken fibroblast growth factor receptor
CH-DOX	Chitosan hydrogel containing doxorubicin
CPT	11, irinotecan
CR	Complete response
CRp	Remission with incomplete platelet recovery
CT	Chemotherapy
CTL	Cytotoxic T lymphocytes
CY	Cyclophosphamide
DC	Dendritic cells
DFS	Disease-free survival
DOX	Doxorubicin

T. Zhang · D. Herlyn (✉)
The Wistar Institute, 3601 Spruce Street,
Philadelphia, PA 19104, USA
e-mail: dherlyn@wistar.org

EBV	Epstein Barr virus
EGF-R	Epidermal growth factor receptor
EGP40	Epithelial glycoprotein 40
GITR	Glucocorticoid-induced TNFR family-related receptor
HPV	Human papilloma virus
i.d.	Intradermally
IFA	Incomplete Freund's adjuvant
IL	Interleukin
i.m.	Intramuscularly
i.p.	Intraperitoneally
IT	Immunotherapy
i.v.	Intravenously
LAMP	Lysosome-associated membrane protein
mAb	Monoclonal antibody
MCL	Mantle cell lymphoma
MHC	Major histocompatibility complex
MR	Mixed response
MTX	Methotrexate
MVA	Modified vaccinia Ankara
NA	No assessment
NR	No response
NT	Not tested
OR	Objective (>50%) regression
PD	Progressive disease
pfu	Plaque forming units
PKA	Protein kinase
PMT	Progression median time
p.o.	Per os
PR	Partial response
PSA	Prostate-specific antigen
rF	Recombinant fowlpox virus
rV	Recombinant vaccinia virus
s.c.	Subcutaneously
SD	Stable disease
SINCP	Sindbis virus
TAP	Tumor associated peptides
TAX	Paclitaxel
TGF	Transforming growth factor
TIL	Tumor infiltrating lymphocytes
TMTX	Antifolate trimetrexate
Treg	Regulatory T cells
TS-1	5-FU derivative
VBL	Vinca alkaloid vinblastine
VEGFR	Vascular endothelial growth factor receptor
VP-16	Topoisomerase II inhibitor etoposide
VRP	Venezuelan equine encephalitis virus replicon particles

Introduction

Combination between immunotherapy and chemotherapy has long been viewed as incompatible as chemotherapy, especially at high doses meant to increase the anti-tumor efficacy, has induced immunosuppression. Possible mechanisms of immune suppression by chemotherapy are induction of lymphopenia, immunosuppressive cytokines, immune tolerance by high doses of antigens released by the dying tumor cells, and inhibition of immune effector cell function [3, 90, 94, 155]. However, in the 1960s, Mihich already demonstrated in murine leukemia model that the curative effects of chemotherapy are due to the induction of immune response directed against the tumor cells [91–93]. Immunoaugmentation has also been shown in later studies following chemotherapy with some drugs at low doses [3, 47, 90, 94, 155]. Treatment of cytotoxic T lymphocytes (CTL) with certain chemotherapeutic drugs enhanced their capacity to lyse Epstein Barr virus (EBV)-transformed lymphocytes, whereas other drugs showed inhibitory activities [86]. Experimental evidence has shown that direct effects of chemotherapy on tumor and host environment, which are discussed in detail below, may counteract its immunosuppressive effects, leading to enhancement of anti-tumor immune response.

We have reviewed here experimental and clinical approaches to combining active specific immunotherapy, or adoptive antibody or cellular immunotherapy with chemotherapy in the treatment of cancer. Most of the previous review articles did not cover combination of adoptive antibody or cellular immunotherapy with chemotherapy in preclinical and clinical studies and, in contrast to our article, none (including also articles on combined active specific immunotherapy and chemotherapy) describe experimental details, which are important to better understand differences in the results obtained with similar combination therapies by different investigators [3, 18, 21, 32, 45, 48, 58, 73–75, 77, 83, 90, 95, 96, 101, 117, 123, 132, 137, 143, 144]. The experimental approaches in this review include only studies which are carefully controlled to demonstrate that a combination of both therapies is superior to the use of either therapy alone. Clinical trials with combination therapies are also included in this review although they were not randomized and have not yet reached phase III. This review article does not include studies in which non-specific immune modulators such as cytokines were combined with chemotherapeutic agents. These studies have recently been reviewed by Zitvogel et al. [155].

Pre-clinical and clinical studies of combined mAb IT and CT

MAb therapy, which has long been viewed as unsuccessful, has been greatly rejuvenated by its combination with chemotherapeutics. Naked and radiolabelled mAb in combination with chemotherapeutics, or mAb linked to drugs have been used for the treatment of various malignancies in mice and cancer patients (Tables 1, 2). In mice, the anti-tumor effects of these combination therapies were significantly greater compared to either therapy alone. Of note, in each of the experimental studies (Table 1), significant effects were seen against established tumors. In cancer patients, impressive clinical responses were reported with combination therapies targeting specifically CD33 in leukemias, CD20 in B cell lymphomas, HER-2 in breast carcinomas, and epidermal growth factor receptor (EGF-R) in head and neck carcinomas (Table 2). The possible mechanisms underlying therapeutic effects of this combination therapy are discussed below.

Pre-clinical and clinical studies of combined active specific IT and CT

The possible mechanisms underlying synergistic effects of active specific IT and CT are quite well understood, but selection of optimal dose of chemotherapy and timing of administration of the two therapies remain a challenge (see below). Various forms of vaccine delivery, such as irradiated tumor cells, tumor cell extract, tumor proteins or antigens expressed in naked plasmids or viral vectors have been used in combination with chemotherapeutics in several tumor models in mice (Table 3). In some of these studies, combination therapy was able to inhibit growth of established tumors [2, 19, 28, 46, 49, 61, 65, 67, 69, 70, 72, 76, 109, 130, 140, 141, 153, 154]. In clinical trials in which combined vaccine/chemotherapy was compared with either therapy or IT alone, promising clinical responses have been reported. Thus, the number of glioblastoma patients demonstrating 2 year disease-free survival was increased after treatment with dendritic cells (DC) loaded with tumor peptides or lysates, followed by chemotherapy with Temozolomide and BCNU as compared to treatment with either therapy alone [148] (Table 4). Clinical response rates of prostate cancer patients were increased following immunization with tumor peptides in combination with chemotherapy (Estramustine phosphate) as compared to IT alone [103] (Table 4). In another trial in prostate cancer patients, median time to tumor progression was increased after combination therapy (recombinant vaccinia virus expressing prostate specific antigen, followed by doxorubicin), compared to IT alone [6] (Table 4).

Pre-clinical and clinical studies of adoptive lymphocyte or active specific IT in combination with lymphodepletion by CT

The combination of adoptive lymphocyte IT with lymphodepletion by CT in patients with refractory metastatic (stage IV) melanoma has resulted in remarkable clinical response rates of approximately 50% [44] (Table 5), whereas clinical response rates with various CTs or adoptive lymphocyte transfer alone usually ranged between 10 and 34% in historical control patients [128, 129]. Various mechanisms may underly the synergistic effects of lymphodepletion on adoptive lymphocyte IT (see below). Lymphodepletion also has been combined with both active specific and adoptive lymphocyte IT in six metastatic melanoma patients. Thus, each patient received all three therapies [5] (Table 5). Only one of six patients showed a partial response to this combination therapy and it is unclear which form of therapy this response may be attributed to.

Treatment of well established tumors in mice with a chemotherapeutic drug, followed by adoptive lymphocyte IT resulted in tumor regression [152] (Table 5). Interestingly, synergism between the two therapies was dependent on the tumor microenvironment (see below).

Discussion and conclusions

The major possible direct effects of chemotherapy on tumor and/or host environment, which provide a rationale for combining CT with active and/or adoptive cellular IT, are:

Induction of tumor cell death

In the early studies by Bonmassar, it was shown that various types of immunogenic modification of tumor cells might occur in tumor-bearing hosts after treatment with drugs in vivo [15, 52, 68, 102, 125]. The molecular mechanism of drug-mediated immunogenic changes could be related to somatic mutations [51, 56]. Notably, chemotherapy of tumor-bearing mice and breast cancer patients was followed by induction of immune responses to the tumors [66, 97, 104, 109, 125]. Induction of necrosis and/or apoptosis in tumor cells in vitro has frequently been shown to increase their immunogenicity in vivo [3, 20, 54, 78, 90, 94, 107, 124]. Most likely, necrotic or apoptotic tumor cells induced by chemotherapy were phagocytosed by antigen-presenting cells (APC), presented to immune lymphocytes, followed by the stimulation of an anti-tumor responses in the lymphocytes [3, 55, 79, 90, 94]. Through induction of cell death by chemotherapeutics, a tumor

Table 1 Effect of combined mAb IT and CT on tumor growth and/or survival in mice

Tumor type	mAb IT				CT				Temporal relationship between IT and CT	Effect of combined therapy on		Possible mechanism of tumor growth inhibition		Ref.
	Designation (Specificity)	Dose	Frequency of application	Route of administration	Designation	Dose	Frequency of application	Route of administration		Growth of established tumor	Survival after tumor challenge	IT	CT	
Human colon carcinoma	^{131}I -A33 (A33 Ag)	0.1 mCi/mouse	1 ×	i.v.	5-FU or 5-FU + leucovorin, DOX, or carmustine	0.75 - 75 mg/kg	2 x or 5 x	i.p.	IT and CT simultaneously	Inhibition	NT	Radiation	Apoptosis	[142]
Human acute lympho-blastic leukemia	CMC-544 conjugated to caliche-amicin (CD22)	80-160 µg/kg	3 x	i.p.	Calicheamicin	160 µg/kg	3 x	i.p.	IT and CT simultaneously	Inhibition	Enhance-ment	Tumor-targeted delivery of cytotoxic agent	Apoptosis	[37, 38, 40, 41]
Human B-cell non-Hodgkin's lymphoma	CMC-544 conjugated to caliche-amicin (CD22) Rituximab (CD20)	80-160 µg/kg 20 mg/kg	3 x 3 x	i.p. i.p.	Calicheamicin	80-160 µg/kg	3 x	i.p.	IT and CT simultaneously	Inhibition	Enhance-ment	Tumor-targeted delivery of cytotoxic agent ADCC and CDC	Apoptosis	[39]
Murine meso-thelioma	FGK45 (CD40)	100 µg/ mouse	3 x	i.v.	Gemcitabine	120 µg/g	5 x	i.p.	IT and CT simultaneously	Inhibition	Enhance-ment	Activation of DC	Apoptosis, activation of $CD4^+$ and $CD8^+$ T cells	[105]
Murine sarcoma cells (AG104)	Anti-CD137 (CD137)	200 µg/mouse	2 x	i.p.	TMTX	17.5 mg/kg	5 x	i.p.	CT 4 days after IT	Inhibition	Enhance-ment	Activation of T-cell responses	Apoptosis	[89]
Human colon carcinoma	^{131}I-F(ab') 2-35, CE25, B17 and B93 (CEA)	800 or 1600 µCi /mouse	1 or 2 ×	i.v.	5-FU	40 mg/kg	5 ×	i.p.	CT before, simultaneously and after IT	Inhibition	NT	Radiation	Apoptosis and radiosensiti zation	[22]
Human breast cancer	90Y-Chimeric L6 (undefined integral membrane glycoprotein)	260 µCi/ mouse	1 ×	i.v.	TAX	600 µg/ mouse	1 ×	i.p.	CT 1 day after IT	Inhibition	NT	Radiation	Apoptosis	[34]
Human breast adenocarcinoma cell & squamous carcinoma cell	528 & 225 (EGFR)	1 mg/ mouse	10 x	i.p.	DOX	50-100 µg/ mouse	2 x	i.p.	IT and CT simultaneously	Inhibition	Enhance-ment	Blockade of EGF-R activation	Apoptosis, increase in EGF-R expression	[9]
Human colon carcinoma	C225 (EGF-R)	0.25 mg/kg	10 ×	i.p.	8-Cl-cAMP	0.5 mg/ mouse	10 ×	i.p.	IT and CT simultaneously	Inhibition	Enhance-ment	Blockade of EGF-R activation	Inhibition of cAMP-dependent PKAI and TGF-α	[24]
Human colon carcinoma	C225 (EGF-R)	0.25 mg/kg	10 ×	i.p.	Topotecan	2 mg/kg	4 x	i.p.	IT and CT simultaneously	Inhibition	Enhance-ment	Blockade of EGF-R activation	Inhibition of topo-isomerase	[23]
Human colon carcinoma	C225 (EGF-R)	1 mg /mouse	14 x	i.p.	Irinotecan	100 -150 mg/kg	7 x	i.p.	CT 3 days before IT	Inhibition	NT	Blockade of EGF-R activation	Apoptosis	[121]
Human colon carcinoma	C225 (EGF-R)	1 mg /mouse	7 x	i.p.	Oxalipatin	10 mg /kg	1 x	i.v.	IT and CT simultaneously	Inhibition	NT	Blockade of EGF-R activation	Apoptosis	[7]
Human epidermoid carcinoma	225 and 528 (EGF-R)	1 mg /mouse	8 x	i.p.	*cis*-diamminedich loroplatinum	6 mg/kg	2 x	i.p.	IT and CT simultaneously	Inhibition	Enhance-ment	Blockade of EGF-R activation	Apoptosis	[50]
Human pancreatic cancer	C225 (EGF-R)	1 mg /mouse	2 x	i.p.	Gemcitabine	250 mg/kg	2 x	i.p.	IT 1 day before CT	Inhibition	NT	Blockade of EGF-R activation	Apoptosis	[17]
Human ovarian cancer	^{131}I-323/A3 (EGP40)	200 µCi/ mouse	2 ×	i.v.	CDDP	4 mg/kg	2 ×	i.v.	CT 1 day after IT	Inhibition	NT	Radiation	Apoptosis	[71]
Human breast cancer	Herceptin (HER2)	0.3 mg/kg	10 x	i.p.	TAX DOX	10 mg/kg 10 mg/kg	2 × 1 ×	i.v. i.p.	TAX on days 1 and 4 of IT DOX on day 1 of IT	NT	Enhance-ment	HER-2 downre-gulation by Ab, leading to cell growth inhibition by increased suscepti-bility to CT; or HER-2	Inhibition of cell division by tubulin polymerizat ion	[8]

Table 1 continued

Tumor type	mAb IT				CT				Temporal relationship between IT and CT	Effect of combined therapy on		Possible mechanism of tumor growth inhibition		Ref.
	Designation (Specificity)	Dose	Frequency of application	Route of administration	Designation	Dose	Frequency of application	Route of administration		Growth of established tumor	Survival after tumor challenge	IT	CT	
												upregulation by TAX leading to Herceptin-mediated apoptosis of tumor cells		
Human breast cancer	4D5 (HER-2)	1 or 3 mg/kg	3 ×	i.p.	CDDP	0.25 or 0.75 mg/kg	1 ×	i.p.	CT immediately after IT	Inhibition	NT	HER-2 downregulation, leading to cell growth inhibition by increased susceptibility to CT	Apoptosis	[116]
Human breast cancer	Rhu mAb (HER2)	4-10 mg/kg	1 or 2 ×	i.p.	MTX VP-16 5-FU VBL DOX CY TAX	2 mg/kg 20 mg/kg 16 mg/kg 0.8 mg/kg 5 mg/kg 80 mg/kg 15 mg/kg	2 x 2 x 2 x 2 x 1 x 3 x 3 x	i.p.	IT and CT simultaneously	Inhibition	NT	HER-2 downregulation, leading to cell growth inhibition by increased susceptibility to CT	Apoptosis	[111]
Human prostate cancer	Herceptin (HER2-neu)	20 mg/kg	6 x	i.p.	TAX	6.25 mg/kg	15 x	s.c.	IT and CT simultaneously	Inhibition	NT	HER-2 downregulation, leading to cell growth inhibition by increased susceptibility to CT	Inhibition of cell division by tubulin polymerization	[1]
Human lung carcinoma	^{131}I-Po66 (undefined intracellular Ag)	250 µCi / mouse	3 ×	i.v.	DOX	8 mg/kg	2 ×	i.v.	IT 1 day after CT	Inhibition	NT	Radiation	Apoptosis and enhanced accessibility of Ag for mAb	[35, 36]
Human ovarian cancer	^{90}Y-DOTA 776.1 (CA 125)	50 or 150 µCi / mouse	1 x	i.v.	TAX	10 mg/kg	1 x	i.p.	IT 1 day after CT IT 1 day before CT	Inhibition Inhibition	NT NT	Radiation	Apoptosis	[88]
Human pancreatic cancer	^{90}Y-PAM4 (MUC1)	25 µCi / mouse	3 ×	i.v.	Gemcitabine	1000 mg/m^2	9 ×	i.p.	IT and CT simultaneously	Inhibition	Enhancement	Radiation	Apoptosis, radiosensitization of tumor cells	[57]

5-FU 5-fluorouracil, *Ag* antigen, *CDDP* cisplatin, *CEA* carcinoembryonic antigen, *CT* chemotherapy, *CY* cyclophosphamide, *DC* dendritic cells, *DOX* Doxorubicin, *EGF-R* epidermal growth factor receptor, *EGP40* epithelial glycoprotein 40, *i.p.* intraperitoneally, *IT* immunotherapy, *i.v.* intravenously, *mAb* monoclonal antibody, *MTX* Methotrexate, *NT* not tested, *PKA* protein kinase, *s.c.* subcutaneously, *TAX* paclitaxel, *TGF* transforming growth factor, *TMTX* antifolate trimetrexate,*VBL* vinca alkaloid vinblastine, *VP-16* Topoisomerase II inhibitor etoposide

could become its own cellular vaccine by crosspresentation of the apoptotic cells to APC, or induction of pro-inflammatory mediators such as heat shock proteins or interleukin (IL)-6, followed by crosspriming of immune effector cells [80, 145]. Although different chemotherapeutic agents may kill tumor cells through an apparently homogeneous apoptotic pathway, they may differ in the mechanism underlying the induction of immunogenic cell death. Thus, the chemotherapeutic agent anthracyclin induced an immune response to tumor cells only when apoptosis was preceded by translocation of calreticulin to the plasma membrane. Blockade or knock-down of calreticulin

Table 2 Clinical trials of combined mAb IT and CT

Tumor type	mAb IT				CT				Temporal relationship between IT & CT	No. of patients	Clinical outcome (No. of patients)	Possible mechanism of therapeutic effect		Ref.
	Designation (specificity)	Dose	Frequency of application	Route of administration	Designation	Dose	Frequency of application	Route of administration				mAb	CT	
Pancreatic carcinoma	17-1A	400 mg /patient	1 x	i.v.	5-FU	600 mg/m^2	4 ×	i.v.	CT 1 day after IT	8	PR: 2 NR: 6	ADCC, idiotypic network	Apoptosis	[110]
					adriamycin	30 mg/m^2	2 x	i.v.						
					mitomycin	10 mg/m^2	1 x	i.v.						
B-cell lymphoma	Rituximab (CD20)	375 mg/m^2	6 ×	i.v.	CY	750 mg/m^2	6 ×	i.v.	CT 7 days after IT	38	CR: 22 PR: 16	ADCC, CDC, apoptosis by crosslinking of CD20	Apoptosis	[29-31]
					Dox	50 mg/m^2	6 ×	i.v.						
					vincristine	1.4 mg/m^2	6 ×	i.v.						
					prednisone	100 mg/m^2	30 ×	p.o.						
B- cell lymphoma	^{131}I-tositumomab (CD20)	1.7 mg/kg (20-27 Gy)	1-4 x	i.v.	Etoposide	60 mg/kg	1-4 x	i.v.	CT 2 days after radio-IT	31	CR: 24 PR: 3 SD: 2 PD: 1	Radiation, apoptosis by crosslinking of CD20	Apoptosis	[118]
					CY	100 mg/kg	1-4 x	i.v.						
B-cell lymphoma	Rituximab (CD20)	375 mg/m^2	6 ×	i.v.	CY	750 mg/m^2	6-8 ×	i.v.	IT and CT simulta-neously	3	CR: 3	ADCC, CDC, apoptosis by crosslinking of CD20	Apoptosis	[16]
					Dox	50 mg/m^2	6 -8 ×	i.v.						
					vincristine	1.4 mg/m^2	6 -8 ×	i.v.						
					prednisone	100 mg/m^2	30 -40 ×	p.o.						
					MTX	15 mg/m^2	8 x	i.v.						
Non-Hodgkin's lymphoma	Rituximab (CD20)	375 mg/m^2	4 ×	i.v.	CY	750 mg/m^2	3 ×	i.v.	CT 1 day after IT	18	CR: 7 PR: 10 PD: 1	ADCC, CDC, apoptosis by crosslinking of CD20	Apoptosis, mobilization of peripheral blood stem cells	[147]
					Dox	50 mg/m^2	3 ×	i.v.						
					vincristine	1.4 mg/m^2	3 ×	i.v.						
					prednisone	100 mg/m^2	15 ×	p.o.						
					Cytosine arabinoside	2000 mg/m^2	4 x	i.v.						
					mitoxan-trone	10 mg/m^2	2 x	i.v.						
Non-Hodgkin's Lymphoma	Rituximab (CD20)	375 mg/m^2	6 ×	i.v.	CY	750 mg/m^2	6-8 ×	i.v.	CT 1 day after IT	33	CR: 20 PR: 11 PD: 2	ADCC, CDC, apoptosis by crosslinking of CD20	Apoptosis	[146]
					Dox	50 mg/m^2	6-8 ×	i.v.						
					vincristine	1.4 mg/m^2	6-8 ×	i.v.						
					prednisone	100 mg/m^2	30-40 ×	p.o.		202	CR: 152 PR: 15 SD: 2 PD: 31 NA: 2			[25, 26]
Non-Hodgkin's Lymphoma	^{131}I-tositumomab (CD20)	5-10 mCi	2 x	i.v.	CY	750 mg/m^2	6 ×	i.v.	CT 30 to 60 days after Radio-IT	90	CR: 62 PR: 20 SD: 2 NA: 6	Radiation, apoptosis by crosslinking of CD20	Apoptosis	[119, 120]
					Dox	50 mg/m^2	6 ×	i.v.						
					vincristine	1.4 mg/m^2	6 ×	i.v.						
					prednisone	100 mg/m^2	30 ×	p.o..						
MCL	^{131}I-tositumomab (CD20)	5-10 mCi (1.7 mg/kg)	2 x	i.v.	Etoposide	30-60 mg/kg	1 x	i.v.	CT 10 days after IT	11	CR: 8 PR: 1 NR: 2	Radiation, apoptosis by crosslinking of CD20	Apoptosis	[59]
					CY	60-100 mg/kg	1 x	i.v.						
Acute myeloid leukemia	CMA-676 linked to calicheamicin (CD33)	9 mg/m^2	2 x	i.v.	Caliche-amicin	9 mg/m^2	2 x	i.v.	IT and CT simulta-neously	142	CR: 23 CRp: 19 NR: 100	Tumor-targeted delivery of cytotoxic agent	Apoptosis	[122, 135, 136]
Acute myeloid leukemia	Gemtuzumab linked to calicheamicin (CD33)	9 mg/m^2	2 x	i.v.	Caliche-amicin	9 mg/m^2	2 x	i.v.	IT and CT simulta-neously	101	CR: 13 CRp: 15 NR: 73	Tumor-targeted delivery of cytotoxic agent	Apoptosis	[81]
Head and neck cancer	C225 (EGFR)	250 and 400 mg/m^2	6 x	i.v.	Cisplatin	100 mg/m^2	2 x	i.v.	CT 1 day after IT	9	CR: 2 PR: 4 PD: 3	Inhibition of tumor cell proliferation by EGFR blockade.	Apoptosis	[134]

Table 2 continued

Tumor type	mAb IT				CT				Temporal relationship between IT & CT	No. of patients	Clinical outcome (No. of patients)	Possible mechanism of therapeutic effect		Ref.
	Designation (specificity)	Dose	Frequency of application	Route of administration	Designation	Dose	Frequency of application	Route of administration				mAb	CT	
Small cell lung cancer, head and neck cancer	C225 (EGFR)	200 and 400 mg/m^2	12 x	i.v.	Cisplatin	60 mg/m^2	3 x	i.v.	CT 1 day after IT	22	CR: 1 PR: 2 SD: 11 PD: 8	EGFR blockade	Apoptosis	[10]
Squamous cell carcinoma of the head and neck	Cetuximab (EGFR)	250 and 400 mg/m^2	3-4 x	i.v.	Cisplatin Carboplatin	60 mg/m^2 250 mg/m^2	2-4 x 2-4 x	i.v. i.v.	CT 1 hour after IT	96	PR: 10 SD: 41 PD: 27 NA: 14 Missing: 4	EGFR blockade	Apoptosis	[11].
Pancreatic cancer	Cetuximab (EGFR)	250 and 400 mg/m^2	7-90 x	i.v.	Gemcitabine	1000 mg/m^2	7-90 x	i.v.	IT and CT simultaneously	41	PR: 5 SD: 26 PD: 6 NA: 4	EGFR blockade	Apoptosis	[149]
Pancreatic cancer	Matuzumab (EGFR)	400 or 800 mg/m^2	8 x	i.v.	Gemcitabine	1000 mg/m^2	6 x	i.v.	IT and CT simultaneously	12	PR: 3 SD: 5 PD: 4	EGFR blockade	Apoptosis	[60]
Breast cancer	Trastuzumab/ Herceptin (HER-2)	100 or 250 mg /patient	9 x	i.v.	CDDP	75 mg/m^2	3 ×	i.v.	CT 1 day after IT	37	PR: 9 SD: 9 PD: 19	Inhibition of tumor cell proliferation by down-regulation of HER-2 receptor	Apoptosis	[112, 113]
Breast cancer	Trastuzumab (HER-2)	4 or 2 mg/kg	40 x	i.v.	Dox CY Epirubicin Paclitaxel	60 mg/m^2 600 mg/m^2 75 mg/m^2 175 mg/m^2	80 x 80 x 80 x 80 x	i.v. i.v. i.v. i.v.	CT 7 days after IT	235	CR: 18 PR: 100 PD: 117	Inhibition of tumor cell proliferation by down-regulation of HER-2 receptor	Apoptosis	[138]
Breast cancer	Trastuzumab (HER-2)	4 or 2 mg/kg	< 52 x	i.v.	Paclitaxel Dox	80-150 mg/m^2 60 mg/m^2	12 x 3 x	i.v. i.v.	IT 1 day or 10 weeks after CT	32	CR: 5 PR: 23 SD: 4	Inhibition of tumor cell proliferation by down-regulation of HER-2 receptor	Apoptosis	[14]
Breast cancer	Trastuzumab (HER-2)	8 or 6 mg/kg	8 x	i.v.	MTX CY	2.5 mg 50 mg	48 x 180 x	p.o. p.o.	IT and CT simultaneously	22	PR: 4 SD: 10 PD: 8	Inhibition of tumor cell proliferation by down-regulation of HER-2 receptor	Apoptosis; low dose CY and MTX reduction of VEGF level	[108]
Breast cancer	Trastuzumab (HER-2)	4 or 2 mg/kg	52 x	i.v.	Dox CY Paclitaxel	60 mg/m^2 600 mg/m^2 175 mg/m^2	4 x 4 x 4-12 x	i.v. i.v. i.v.	IT and CT simultaneously	1679/1672[a]	67.1%/85.1%[a] 4 yrs DFS	Inhibition of tumor cell proliferation by down-regulation of HER-2 receptor	Apoptosis	[126]
Breast cancer	Trastuzumab (HER-2)	8 or 6 mg/kg	35 x	i.v.	Combination with DOX, CY, 5-FU, MTX, epirubicin, paclitaxel, Taxane, docetaxel	50-720 mg/m^2	> 4 x	p.o. or i.v.	NA	1693/1694[a]	77.4%/85.8%[a] 2 yrs DFS	Inhibition of tumor cell proliferation by down-regulation of HER-2 receptor	Apoptosis	[114]
Breast cancer	Trastuzumab (HER-2)	8 or 6 mg/kg	52 x	i.v.	Combination with DOX, CY, 5-FU, MTX, epirubicin, paclitaxel, Taxane, docetaxel	50-720 mg/m^2	> 4 x	p.o. or i.v.	NA	1698/1703[a]	74.3%/80.6%[a] 3 yrs DFS	Inhibition of tumor cell proliferation by down-regulation of HER-2 receptor	Apoptosis	[139]

Table 2 continued

Tumor type	mAb IT				CT				Temporal relationship between IT & CT	No. of patients	Clinical outcome (No. of patients)	Possible mechanism of therapeutic effect		Ref.
	Designation (specificity)	Dose	Frequency of application	Route of administration	Designation	Dose	Frequency of application	Route of administration				mAb	CT	
Breast cancer	Trastuzumab (HER-2)	4 or 2 mg/kg	30-200 x	i.v.	Dox	100 mg/m^2	> 6 x	i.v.	NA	92	CR: 6 PR: 50 SD: 25 PD: 11	Inhibition of tumor cell proliferation by down-regulation of HER-2 receptor	Apoptosis	[87]

[a] CT/CT + IT

5-FU 5-fluorouracil, *ADCC* antibody-dependent cell-mediated cytotoxicity, *CDC* completment-dependent cytotoxicity, *CDDP* cisplatin, *CR* complete response, *CRp* remission with incomplete platelet recovery, *CT* chemotherapy, *CY* cyclophosphamide, *DFS* disease-free survival, *Dox* Doxorubicin, *IT* immunotherapy, *i.v.* intravenously, *MCL* Mantle cell lymphoma, *MTX* methotrexate, *NA* no assessment, *NR* no response, *PD* progressive disease, *p.o.* per os, *PR* partial response, *SD* stable disease

suppressed the phagocytosis of anthracyclin-treated tumor cells by dendritic cells and abolished their immunogenicity in mice [3, 90, 106, 107].

In principle, any therapy that delivers higher levels of cross-presented tumor antigens to the draining lymph nodes could synergize with immunotherapy. Thus, anti-tumor immunity induced by apoptotic tumor cells following chemotherapy can be boosted by active specific immunotherapy (see Tables 3, 4).

Elimination of regulatory T (Treg) cells

Cyclophosphamide (Cy) may down regulate the activity of Treg, especially when used in low doses [3, 82, 84, 90, 94, 99], whereas high doses may have direct tumor-cytotoxic effects [97–99]. Cy has been widely used in conjunction with active specific IT to enhance anti-tumor immune responses by down regulation of Treg, and this combination therapy has been pioneered by Berd et al. [12, 13] (Table 3).

Enhancement of tumor cell sensitivity to lysis by CTL

Active specific immunotherapy often induces low avidity CTL which do not effectively lyse tumors. However, when melanoma cells were treated with chemotherapeutic agents in vitro, they became highly sensitive to lysis by low avidity CTL. Cytotoxic drug-mediated sensitization primed both perforin/granzyme and Fas-mediated killing by the CTL [151]. In a related study, treatment of cancer cells with 5-aza-2′-deoxycytidine restored the expression of major histocompatibility complex (MHC) class I molecules and cancer testis antigens on tumor cells, rendering the tumor cells susceptible to CTL attack [133].

In a reverse manner, immunotherapy may directly modulate the tumor's sensitivity to chemotherapy:

a. Monoclonal antibody Rituximab, used for passive immunotherapy of B cell lymphoma and non-Hodgkin's lymphoma cancer patients, has reverted chemoresistance in B cell lymphoma cell lines to chemosensitivity [33]. Chemosensitization of tumor cells was due to downregulation of TNF-alpha secretion, but not to downmodulation of either the MDR-1 or bcl-2 gene products. Also, Her2-neu downregulation by mAb Herceptin increased tumor cell sensitivity to cisplatin by decreasing DNA repair activity following cisplatin-induced DNA damage [62, 115].
b. In several clinical trials, IT was followed by salvage CT [4, 6, 103, 148] (Table 4). Patients treated with this combination therapy showed higher clinical response rates as compared to historical controls treated with CT alone, although larger randomized and carefully controlled trials must be conducted to convincingly demonstrate beneficial effects of combination therapies. It is not known whether in the trials mentioned above IT "conditioned" the tumor to destruction by CT as shown for combinations of mAb and CT [33, 62, 115]. Gabrilovich [53] suggests that the anti-tumor effects of IT followed by CT are exerted independently by the two therapies and synergistic effects of this combination therapy may be dependent on optimal timing and scheduling of the two therapies. Specifically, CT may need to be started quickly after the administration of IT as anti-tumor immune responses generated by IT can not be sustained for a long period of time in cancer patients [53]. On the other hand, studies in tumor-bearing experimental animals have shown that delaying CT after IT increases the anti-tumor efficacy of this combined treatment, evidently through inhibition of vaccine-induced regulatory T cells by the chemotherapeutic drug [28] (Table 3).

Table 3 Effect of combined active specific IT and CT on tumor growth and/or survival in experimental animals

Tumor type	Vaccine IT				CT				Temporal relationship between IT and CT	Effect of combined therapy on		Possible mechanism of tumor growth inhibition		Ref.
	Composition	Dose	Frequency of application	Route of administration	Designation	Dose	Frequency of application	Route of administration		Growth of established tumor	Survival after tumor challenge	IT	CT	
Murine AML	Irradiated, B7.1-transduced AML cells	10^5 cells/mouse	1 ×	i.v.	Ara-C	200 mg/kg	3 ×	i.p.	IT 8 days after CT	NT	Enhancement	$CD8^+$ CTL response against AML cells	Apoptosis	[46]
Human breast carcinoma-derived Ehrlich Ascites Carcinoma, EAC)	Irradiated EAC cells or cell extract	4×10^5 cells/10g body weight	5 x	i.p.	Derivatives and analogs of glutamine and glutamic acid	50 mg/kg	5 x	i.p.	IT and CT simultaneously	NT	Enhancement	NT	Apoptosis	[130]
	Irradiated EAC cells or cell extract	4×10^5 cells/10g body weight	5 x	i.p.	Etoposide	2.5 mg/kg	5 x	i.p.	IT and CT simultaneously	NT	Enhancement	NT	Apoptosis	
Murine breast cancer	Ad-sig-TAA/ecd CD40L infected DCs	5×10^5/mouse	1 x	i.t.	5-FC	500 mg/kg	10 x	i.p.	IT 3 days after CT	Inhibition	Enhancement	Tumor-specific CTL	Apoptosis	[2]
Murine breast cancer	SINCP-HER2/neu plasmid	100 µg/mouse	3 x	i.m.	DOX	5 mg/kg	1 x	i.v.	IT 1 day after CT	Inhibition	NT	NT	Apoptosis	[49]
	SINCP-HER2/neu plasmid	100 µg/mouse	3 x	i.m.	Paclitaxel	25 mg/kg	1x	i.p.	IT 1 day after CT	No effect	NT	NT	Apoptosis	
	VRP-HER2/neu	10^6 infectious units/mouse	3 x	Foot pad	DOX	5 mg/kg	1 x	i.v.	IT 1 day after CT	Inhibition	NT	$CD8^+$ CTL response	Apoptosis, enhancement of immune responses	
	VRP-HER2/neu	10^6 infectious units/mouse	3 x	Foot pad	Paclitaxel	25 mg/kg	1 x	i.p.	IT 1 day after CT	Inhibition	NT	$CD8^+$ CTL response	Apoptosis, enhancement of immune responses	
Murine breast cancer	Irradiated HER2/neu + GM-CSF transduced 3T3 cells	3×10^6 cells/mouse	8 x	s.c.	DOX	5 mg/kg	2 x	i.v.	IT 1 day after CT	Inhibition	NT	Th1 T-cell response	Apoptosis	[85]
									IT 7 days before CT	Inhibition	NT		Apoptosis	
					Paclitaxel	20 mg/kg	2 x	i.p.	IT 1 day after CT	Inhibition	NT		Apoptosis, enhancement of Th1 T-cell response	
									IT 7 days before CT	Inhibition	NT		Apoptosis, inhibition of Th1 T-cell response	
					CY	100 mg/kg	2 x	i.p.	IT 1 day after CT	Inhibition	NT		Apoptosis, enhancement of Th1 T-cell response	
									IT 7 days before CT	Inhibition	NT		Apoptosis, inhibition of Th1 T-cell response	

Table 3 continued

Tumor type	Vaccine IT				CT				Temporal relationship between IT and CT	Effect of combined therapy on		Possible mechanism of tumor growth inhibition		Ref.
	Composition	Dose	Frequency of application	Route of administration	Designation	Dose	Frequency of application	Route of administration		Growth of established tumor	Survival after tumor challenge	IT	CT	
Canine lymphoma	Irradiated lymphoma cells	10^8 cells/mouse	3 ×	Intralymphatically	Vincristine CY L-asparaginase DOX	0.03 mg/kg 10 mg/kg 400 IU/kg 30 mg/m^2	2 x 2 x 2 x 2 x	i.v. i.v. i.p. i.v.	IT 2 weeks after CT	NT	Enhancement	NT	CY: enhancement of immune response; Other CT agents: Apoptosis	[69]
Murine cervical carcinoma (HPV-16 E7-expressing TC-1)	Vaccinia virus-encoding Sig/E7/LAMP-1	3 x 10^6 pfu/mouse	1 x	i.p.	Epigallocatechin-3-gallate	0.5 mg/ml	5 x	p.o.	CT 3 days after IT	Inhibition	NT	CD8$^+$CTL response	Apoptosis, enhancement of Ag-specific CD8$^+$ CTL response	[70, 140]
	Vaccinia virus-encoding Sig/E7/LAMP-1	3 x 10^6 pfu/mouse	1 x	i.p.	Cisplatin	2.5 mg/kg	1 x	i.v.	CT 3 days after IT	Inhibition	NT	CD8$^+$CTL response	Apoptosis, enhancement of Ag-specific CD8$^+$ response	
	Vaccinia virus-encoding Sig/E7/LAMP-1	3 x 10^6 pfu/mouse	1x	i.p.	CY	50 mg/kg	1 x	i.v.	CT 3 days after IT	Inhibition	NT	CD8$^+$CTL response	Apoptosis, enhancement of Ag-specific CD8$^+$ CTL response	
	Vaccinia virus-encoding Sig/E7/LAMP-1	3 x 10^6 pfu/mouse	1x	i.p.	DOX	2.5 mg/kg	1 x	i.v.	CT 3 days after IT	Inhibition	NT	CD8$^+$CTL response	Apoptosis, enhancement of Ag-specific CD8$^+$ CTL response	
Murine cervical carcinoma (HPV-16 E7-expressing TC-1)	Vaccinia virus-encoding Sig/E7/LAMP-1	3 x 10^6 pfu/mouse	1 x	i.p.	CHDOX	6 mg/kg	1 x	i.m.	CT 3 days after IT	Inhibition	Enhancement	CD8$^+$CTL response	Enhancement of antitumor immune response via cross-presentation of apoptotic tumor body mediated by caspase activation	[61]
Murine colon carcinoma, fibrosarcoma, hepatoma	Recombinant cFGFR	10 µg/mouse	4 x	s.c.	Gemcitabine	10-20 mg/kg	4 x	i.p.	IT 7 days before CT	Inhibition	Enhancement	Inhibition of tumor angiogenesis by anti-FGFR Ab induction	Apoptosis	[153, 154]
Murine colon or lung carcinoma	Recombinant endoglin	10 µg/mouse	4 x	s.c.	Cisplatin	0.6 mg/kg	4 x	i.p.	IT 7 days before CT	Inhibition	Enhancement	Inhibition of tumor angiogenesis by anti-endoglin Ab induction	Apoptosis	[141]
Murine colon carcinoma	Ad human HER-2/neu	2 x 10^8 pfu/mouse	1x	i.m.	Gemcitabine	60 mg/kg	2x	i.p.	IT 2 days after CT	Inhibition	NT	CD8+ CTL response	Apoptosis, elimination of myeloid-derived suppressor cells	[76]
	Anti-GITR Ab	500 µg/mouse	1x	i.p.	Gemcitabine	60 mg/kg	2 x	i.p.	IT 4 days after CT	Inhibition	NT			
	α galactosylceramide-loaded DC transduced with Ad human HER-2/neu	1 x 10^6 cells/mouse	1 x	i.v.	Gemcitabine	60 mg/kg	1x	i.p.	IT 2 days after CT	Inhibition	NT			

Table 3 continued

Tumor type	Vaccine IT				CT				Temporal relationship between IT and CT	Effect of combined therapy on		Possible mechanism of tumor growth inhibition		Ref.
	Composition	Dose	Frequency of application	Route of administration	Designation	Dose	Frequency of application	Route of administration		Growth of established tumor	Survival after tumor challenge	IT	CT	
Murine glioma	Survivin RNA-transfected DCs	1 x 10^6 cells/mouse	3 x	s.c.	Temozolomide	2.5 mg/kg	5 x	i.p.	IT 7 days after CT	NT	Enhancement	Survivin-specific CTL	Apoptosis, tumor Ag cross-priming	[72, 109]
Mouse leukemia	Neuraminidase-treated leukemia cells + BCG	10^4 cells/mouse	1 ×	i.p.	BCNU	12 mg/kg	1 ×	i.p.	IT 36 hr after CT	NT	Enhancement	Ab-mediated CDC	Apoptosis by down-regulation of Bcl-XL and Bcl-2	[19]
Murine lung carcinoma and hepatoma	Recombinant VEGFR	10 µg/mouse	4 x	s.c.	Gemcitabine	20 mg/kg	4 x	i.p.	IT 7 days before CT	Inhibition	Enhancement	Inhibition of tumor angiogenesis by induction of anti-VEGFR Ab	Apoptosis	[67]
Murine lymphoma cells transduced with HLA-A(*)02.01	Thymidylate synthase peptide + CFA	100 µg/mouse	4 x	s.c.	5-FU	125 mg/mouse	4 x	i.p.	CT 21 days after IT	Inhibition (prophylactic study)	NT	CTL	Apoptosis, enhancement of Ag-specific CTL and Ab-mediated CDC	[27]
Murine lymphoma cells transduced with HLA-A(*)02.01	Thymidylate synthase + CFA	100 µg/mouse	3 x	s.c.	Gemcitabine	100 mg/mouse	3 x	i.p.	CT 5 days after IT	NT	Enhancement	CTL	Apoptosis, enhancement of Ag-specific CTL and inhibition of Treg cells	[28]
					Oxaliplatin	50 mg/mouse	3 x	i.p.						
					Leucovorin	100 mg/mouse	6 x	i.p.	CT 1 day before IT	NT	No effect			
					5-FU	125 mg/mouse	6 x	i.p.						
Rat osteosarcoma	Irradiated mouse B7-1 transduced tumor cells	10^6 cells/mouse	4 ×	i.p.	MTX	40 mg/kg	1 ×	i.p.	CT 4 weeks after IT	Inhibition	Enhancement	Enhancement of TIL and proliferative lymphocytes	Apoptosis	[65]

5-FC 5-fluorocytosine, *5-FU* 5-fluorouracil, *Ab* antibody, *Ad* adenovirus, *Ag* antigen, *AML* acute myelogenous leukemia, *BCG* Bacillus Calmette Guerin, *BCNU* 1, 3-bis-(2-chloroethyl)-1-nitrosourea, *CDC* complement-dependent cytotoxicity, *CFA* complete Freund's adjuvant, *cFGFR* chicken fibroblast growth factor receptor, *CH-DOX* chitosan hydrogel containing doxorubicin, *CT* chemotherapy, *CTL* cytotoxic T lymphocyte, *CY* cyclophosphamide, *DC* dendritic cells, *DOX* Doxorubicin, *GITR* glucocorticoid-induced TNFR family-related receptor, *HPV* human papilloma virus, *i.m.* intramuscularly, *IT* immunotherapy, *LAMP* lysosome-associated membrane protein, *MTX* methotrexate, *NT* not tested, *pfu* plaque forming units, *s.c.* subcutaneously; *SINCP* Sindbis virus, *TIL* tumor infiltrating lymphocytes, *VEGFR* vascular endothelial growth factor receptor, *VRP* Venezuelan equine encephalitis virus replicon particles

The therapeutic induction of lymphopenia has raised considerable interest in the context of adoptive lymphocyte transfer therapies and vaccination of melanoma patients [100]. Transient lymphopenia is thought to enhance the efficiency of these therapies by activating homeostatic mechanisms that stimulate the tumor-reactive effector T cells and by counteracting tumor-induced suppression by mechanisms such as regulatory T cells or other mechanisms. Lymphodepletion also enhances T cell homing into tumor beds and intra-tumoral proliferation of effector cells [42, 44] (Table 5).

In an animal model, synergism between CT and adoptive lymphocyte IT was dependent on the involvement of the tumor microenvironment [152] (Table 5). Thus, treating well established tumors expressing low levels of antigen with a chemotherapeutic drug caused sufficient release of antigen to sensitize stromal cells for destruction by adoptively transferred cytotoxic T cells (CTL), resulting in tumor growth inhibition.

In summary, the demonstration of statistically significant survival enhancement in cancer patients treated in randomized phase III trials with mAb and CT vs patients treated with either therapy alone, raises great expectations for combination therapies consisting of active specific IT or adoptive lymphocyte IT with CT, as suggested by studies in experimental animals.

Table 4 Clinical trials of combined active specific IT and CT

Tumor type	Vaccine IT				CT				Temporal relationship between IT & CT	No. of patients	Clinical outcome (No. of patients)	Possible mechanism of therapeutic effect		Ref.
	Designation (specificity)	Dose	Frequency of application	Route of administration	Designation	Dose	Frequency of application	Route of administration				Vaccine	CT	
Colon cancer	TroVax-MVA (tumor Ag 5T4)	5 x 10^8 pfu	6 x	i.m.	Oxaliplatin 5-FU Folinic acid	350 mg/m^2 400-2400 mg/m^2 350 mg/m^2	12 x 12 x 12 x	i.v. i.v. i.v.	IT 4 days before CT	11	CR: 1 PR: 5 SD: 1 PD: 4	Induction of 5T4-specific IFN-γ and/or Ab responses	Apoptosis, enhancement of Ag-cross-presentation, activation of DCs	[63, 64]
Colon Cancer	Four mixed TAP with IFA	3mg	6 x	s.c.	TS-1	20-80 mg/m^2	28 x	p.o.	IT and CT simultaneously	11	SD: 4 PD: 7	Enhancement of TAP-specific CTL and/or Ab responses	Apoptosis, enhancement of Ag-cross-presentation, inhibition of Treg cells	[131]
Glioblastoma	Autologous DC loaded with peptide from tumor cells or autologous tumor lysate	10-40 x 10^6	3 x	s.c.	Temozolomide BCNU	150-200 mg/m^2 150-200 mg/m^2	312 x 42 x	i.v. i.v.	CT after IT	12/12/12[a]	1/1/5[a] 2-yr DFS	Induction of tumor-reactive CTL	Apoptosis	[148]
Pancreatic cancer	Four mixed TAP with IFA	1-6 mg	8-63 x	s.c.	Gemcitabine	1000 mg/m^2	6-48 x	i.v.	IT and CT simultaneously	13	PR: 2 SD: 7 PD: 4	Enhancement of TAP-specific CTL and/or Ab responses	Apoptosis, enhancement of cellular responses	[150]
Prostate cancer	Four mixed TAP	4-12 mg	> 6 x	s.c.	Estramustine phosphate	140 mg	1080 x	p.o.	IT and CT simultaneously	3/13[b]	PR: 1; PD: 2/PR: 6; PD: 7[b]	Enhancement of TAP-specific IFN-γ and/or Ab responses	Apoptosis	[103]
Prostate cancer	rV-PSA rV-B7.1 rF-PSA	3.51 x 10^8pfu 1.17 x 10^8pfu 1.5 x 10^9pfu	1 x 1 x 1 x	s.c. s.c. s.c.	DOX	30 mg/m^2	4 x	i.v.	CT 1 day after IT	14/14[b]	1.8 /3.2 mo PMT[b]	Induction of PSA-specific IFN-γ responses	Apoptosis	[6]
Small cell lung cancer	P53-transfected DCs	1-5 x 10^6	3 x	i.d.	Carboplatin /VP-16 Cisplatin/ VP-16 Cisplatin/CPT-11	100-200 mg/m^2 30-100 mg/m^2 30-125 mg/m^2	9 x 9 x 9 x	i.v. i.v. i.v.	IT after CT	21	CR: 3 PR: 10 SD: 4 PD: 4	Development of p53-specific IFN-γ responses	Down-regulation of tumor-produced immunosuppressive factors	[4]

[a] CT/IT/CT + IT

[b] IT/IT + CT

Ab antibody, *AML* acute myelogenous leukemia, *BCG* Bacillus Calmette Guerin, *BCNU* 1, 3-bis-(2-chloroethyl)-1-nitrosourea, *CPT-11* irinotecan, *CR* complete response, *CT* chemotherapy, *CY* cyclophosphamide, *DC* dendritic cells, *DFS* disease free survival, *i.d.* intradermally, *IFA* incomplete Freund's adjuvant, *IT* immunotherapy, *MR* mixed response, *MTX* Methotrexate, *MVA* modified vaccinia Ankara, *NR* no response, *NT* not tested, *OR* objective (>50%) regression, *PD* progressive disease, *p.o.* per os, *PMT* progression median time, *PR* partial response, *PSA* prostate-specific antigen, *rF* recombinant fowlpox virus, *rV* recombinant vaccinia virus, *s.c.* subcutaneously, *SD* stable disease, *TAP* tumor associated peptides, *TAX* paclitaxel, *TS-1* 5-FU derivative, *VP-16* etoposide

Table 5 Pre-clinical and clinical studies of combined adoptive lymphocyte or active specific IT and CT

Tumor type	Adoptive and active immunotherapy				CT				Temporal relationship between IT & CT	No. of patients or mice	Clinical outcome (No. of patients or mice)	Possible mechanism of therapy		Ref.
	Designation (specificity)	Dose	Frequency of application	Route of administration	Designation	Dose	Frequency of application	Route of administration				IT	CT	
Murine fibro-sarcoma	Transferred Ag-specific T cells	5 x10^6	1 x	i.v.	Gem-citabine	200 µg/g	1 x	i.p.	IT 2 days after CT	8	Rejec-tion of tumor: 7	Tumor Ag-specific CTL	Apoptosis	[152]
Mela-noma	Autologous antitumor lympho-cytes	2.3-13.7 x 10^{10}	1 x	i.v.	CY Fludarabine	60 mg/kg 25 mg/m^2	2 x 5 x	i.v. i.v.	IT 1 day after CT	13	PR: 6 NR-mixed: 4 NR: 3	Tumor-Ag specific CTL	Depletion of Treg cells; altered homeostasis	[42, 43, 127]
	+ IL-2	720,000 IU/kg	15 x	i.v.										
Mela-noma	Autologous antitumor lympho-cytes	1.0-16.0 x 10^{10}	2 x	i.v.	CY Fludarabine	30-60 mg/kg 25 mg/m^2	2 x 5 x	i.v. i.v.	IT 1 day after CT	35	CR: 3 PR: 15 NR-mixed: 8 NR: 9	Tumor Ag-specific CTL	Depletion of Treg cells; altered homeostasis	[44]
	+ IL-2	720,000 IU/kg	15 x	i.v.										
Mela-noma	Melan-A peptide + CpG + IFA	100 µg 500 µg 300 µl	6 x	s.c.	Busulfan Fludarabine	2 mg/kg 30 mg/m^2	2 x 3 x	p.o. i.v.	IT 3 or 5 days after CT	6	PR: 1 PD: 5	Tumor Ag-specific CTL	Depletion of Treg cells; altered homeostasis	[5]
	Melan-A specific $CD8^+$ T cells	1 x 10^9	1 x	i.v.										

CR complete response, *CT* chemotherapy, *CY* cyclophosphamide, *IFA* incomplete Freund's adjuvant; *i.p.* intraperitoneally, *IT* immunotherapy, *i.v.* intravenously, *NR* no response, *NR-mixed* mixed/no response, *PD* progressive disease, *p.o. per os*, *PR* partial response, *s.c.* subcutaneously

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