2004;12:162–168; Russell W.C., J General Virol 2000;81;2573-2604; Volpers C. and Kochanek S. J Gene Med 2004;6, suppl 1: S164-71; Kootstra N.A. and Verma I.M. Annu Rev Pharmacol Toxicol 2003;43: 413-439).

More than 50 different serotypes of adenoviruses have been found in humans. Serotypes are classified into six subgroups A-F and different serotypes are known to be associated with different conditions, i.e., respiratory diseases, conjunctivitis and gastroenteritis. Adenovirus serotype 5 (Ad5) is known to cause respiratory diseases and it is the most common serotype studied in the field of gene therapy. In the first Ad5 vectors E1 and/or E3 regions were deleted enabling insertion of foreign DNA to the vectors (Danthinne X, Imperiale MJ., Gene Therapy. 2000;7:1707-1714). Furthermore, deletions of other regions as well as further mutations have provided extra properties to viral vectors. Indeed, various modifications of adenoviruses have been suggested for achieving efficient anti-tumor effects.

US2010047208 A1 discloses knob-modified adenovirus vectors, in which the tumor targeting is achieved with a modified hTERT promoter and which can be armed with an immunostimulatory protein, such as GM-CSF.

Still, more efficient and accurate gene transfer as well as increased specificity and sufficient tumor killing ability of gene therapies are warranted. Safety records of therapeutic vectors must also be excellent. The present invention provides a cancer therapeutic tool with these aforementioned properties by utilizing both oncolytic and immunotherapeutic properties of adenoviruses in a novel and inventive way.

Brief description ~~of the invention~~

The object of the ~~invention~~disclosure is to provide novel methods and tools for achieving the above-mentioned properties of adenoviruses and thus, solving the problems of conventional cancer therapies. More specifically, the ~~invention~~disclosure provides novel methods and tools for gene therapy.

The present application describes the construction of recombinant viral vectors, methods related to the vectors, and their use in tumor cells lines, animal models and cancer patients.

In one aspect, there is provided an oncolytic adenoviral vector comprising

1) an adenovirus serotype 5 (Ad5) nucleic acid backbone comprising a modified nucleic acid sequence encoding a modified fiber of the virus, wherein the modification of the fiber is Ad5/3 chimerism, insertion of an integrin binding (RGD) region and/or heparin sulphate binding polylysine modification into the fiber,

2) a nucleic acid sequence encoding a tumor specific human telomerase reverse transcriptase (hTERT) promotor upstream of the E1 region; and

3) a nucleic acid sequence encoding human CD40L in the place of the deleted adenoviral genes *gp19k/6.7K* in the E3 region.

The present ~~invention~~disclosure relates to an oncolytic adenoviral vector comprising

1) an adenovirus serotype 5 (Ad5) nucleic acid backbone comprising a capsid modification, preferably a capsid modification with an adenovirus serotype 3 (Ad3) knob (Ad5/3 capsid chimerism),

2) a nucleic acid sequence encoding a tumor specific human telomerase reverse transcriptase (hTERT) promotor upstream of the E1 region; and

3) a nucleic acid sequence encoding human CD40L in the place of the deleted adenoviral genes *gp19k/6.7K* sequence in the E3 region.

In one aspect, there is provided a cell comprising the adenoviral vector as defined herein.

The present ~~invention~~disclosure further relates to a cell comprising the oncolytic adenoviral vector of the ~~invention~~disclosure.

In one aspect, there is provided~~The present invention also relates to~~ a pharmaceutical composition comprising the adenoviral vector as defined herein~~of the invention~~.

In one aspect, there is provided an oncolytic adenoviral vector or pharmaceutical composition as defined herein, which acts as an *in situ* cancer vaccine.

In one aspect, there is provided the use of an adenoviral vector as defined herein in the manufacture of a medicament for treating cancer in a subject.

The present ~~invention~~disclosure also relates to the adenoviral vector of the ~~invention~~disclosure for treating cancer in a subject.

The present ~~invention~~disclosure also relates to a method of treating cancer in a subject, wherein the method comprises administration of the vector or the pharmaceutical composition of the ~~invention~~disclosure to a subject suffering from cancer, especially from cancer refractory to conventional chemotherapeutic and/or radiation treatments.

In one aspect, there is provided an *in-vitro*~~Furthermore, the present invention also relates to a~~ method of producing CD40L in a cell, wherein the method comprises:

a) carrying a vehicle comprising an oncolytic adenoviral vector as defined herein~~of the invention~~ to a cell, and

b) expressing CD40L of said vector in the cell.

Furthermore, the present ~~invention~~disclosure also relates to a method of increasing tumor specific immune response in a subject, wherein the method comprises:

carrying a vehicle comprising an oncolytic adenoviral vector of the ~~invention~~disclosure to a target cell or tissue,

expressing CD40L of said vector in the cell,

increasing the amount of cytotoxic T cells and/or natural killer cells in said target cell or tissue, and

inducing Th2->Th1 switch for enhanced cytotoxic anti-tumor activity in the tumor microenvironment.

In one aspect, there is provided the use of the oncolytic adenoviral vector as defined herein in the manufacture of a medicament for increasing tumor specific immune response, Th1->Th2 switch or reduction of immunosuppression in a subject.

Still, the present ~~invention~~disclosure also relates to a use of an oncolytic adenoviral vector of the ~~invention~~disclosure for producing CD40L in a cell.

Still, the present ~~invention~~disclosure relates to an oncolytic adenoviral vector of the ~~invention~~disclosure for producing CD40L in a cell.

In one aspect, there is provided the use of the oncolytic adenoviral vector as defined herein in the manufacture of a medicament for producing CD40L in a cell.

In one aspect, there is provided the use of a vehicle comprising an oncolytic adenoviral vector as defined herein in the manufacture of a medicament for producing CD40L in a cell, wherein vehicle is to be carried to the cell, and wherein the CD40L of the vector is to be expressed in the cell.

Still, the present ~~invention~~disclosure also relates to a use of an oncolytic adenoviral vector of the ~~invention~~disclosure for increasing tumor specific immune response in a subject.

Still, the present ~~invention~~disclosure relates to an oncolytic adenoviral vector of the ~~invention~~disclosure for increasing tumor specific immune response in a subject.

The present ~~invention~~disclosure provides a novel tool for the treatment of cancers, especially cancers, which are refractory to or incurable by current therapeutic approaches. Also, restrictions regarding tumor types suitable for treatment remain few compared to many other treatments. In fact all solid tumors may be treated with the proposed ~~invention~~disclosure. The treatment can be given intratumorally, intracavitary, intravenously and in a combination of these. The approach can give systemic efficacy despite local injection. The approach can also eradicate cells proposed as tumor initiating ("cancer stem cells").

Besides enabling the transport of the vector to the site of interest the vector of the ~~invention~~disclosure also assures the expression and persistence of the transgene. The present ~~invention~~disclosure solves a problem related to therapeutic resistance of conventional treatments. Furthermore, the present ~~invention~~disclosure provides tools and methods for selective treatments, without toxicity or damages in healthy tissues. Advantages of the present ~~invention~~disclosure include also different and reduced side effects in comparison to other therapeutics. Importantly, the approach is synergistic with many other forms of therapy including chemotherapy and radiation therapy, and can therefore be used in combination regimens.

Induction of an immune reaction towards cells that allow replication of unarmed adenoviruses is normally not strong enough to lead to development of therapeutic tumor immunity. In order to overcome this weakness, the present ~~invention~~disclosure provides armed adenoviruses with a potent inducer of anti-tumor immunity, CD40L, which moreover induces local apoptosis in the tumor tissue.

Specifically, CD40L together with non-replicative viral vectors has been shown to have synergistic potency to enhance activity of effector cells (CD8+ T-cells) by converting Th2 chemokine patterns of T cells into a Th1 type (Loskog et al 2004, J Immunol 172: 7200-5; Bendriss-Vermare et al 2005, J Leucocyte Biol 78: 954-66). Th2 promotes production of antibodies while Th1 encourages cytotoxicity and the latter may be more advantageous when attempting to target T-cells to kill tumor cells. In this patent specification, it is

shown that this phenomenon is particularly potent in the context of an oncolytic adenovirus as demonstrated by preclinical and human data.

Production of CD40L by an oncolytic adenovirus is also important, because it can recruit natural killer cells to the tumor and enhance their anti-tumor activity (Nakajima et al 1998 J Immunol 161:1901-7). Further, CD40L can enhance the function of antigen presenting cells (Nakajima et al 1998 J Immunol 161:1901-7). Finally, the CD40/CD40L interaction provides powerful inhibitory signals to suppressive cells, such as regulatory T-cells, which can result in potent stimulation of anti-tumor immune reactions (Guiducci et al 2005 Eur J Immunol 35:557–67).

Additionally, the viral replication is restricted to target cells by the use of a powerful transcriptionally targeting promoter, hTERT. The tumor specific promoter hTERT is active in practically all advanced solid tumors, but it can also mediate targeting of oncolytic adenoviruses to putative cancer initiating cells, as has been shown in cancer patient pleural effusion samples (Bauerschmitz et al Cancer Res 2008 68: 5533-9). Clinical data presented here indicates no toxicity for normal tissue stem cells, since no life threatening adverse events occurred.

The present ~~invention~~disclosure achieves cancer therapy, wherein tumor cells are destroyed by virion caused oncolysis combined with various different mechanisms activating human immune response, including proliferation and activation of T-cells, macrophages and dendritic cells (DC), followed by cytokine production, which in turn induces a Th1-type immune reaction for additional stimulation of cytotoxic T-cell attack on the tumor. Additionally, CD40L-induced apoptosis promotes reduction in tumor load.

Compared to adenoviral tools of the prior art, the present ~~invention~~disclosure provides a more simple, more effective, inexpensive, non-toxic and/or safer tool for cancer therapy. Furthermore, helper viruses or co-administration of recombinant molecules are not needed.

The present ~~invention~~disclosure provides a new generation of infectivity enhanced and highly effective adenoviruses that retain the good safety of older viruses but result in higher levels of efficacy. Importantly, the present ~~invention~~disclosure describes oncolytic adenoviruses which provide immunological factors critical with regard to the efficacy of oncolytic viruses.

The novel products of the ~~invention~~disclosure enable further improvements in cancer therapy.