INTERVIEW

Advances in the development of parvovirus-based anti-cancer therapies

Antonio Marchini is a Principal Investigator at the German Cancer Research Center (DKFZ, Heidelberg, Germany) and Head of the Laboratory of Oncolytic Virus Immuno Therapeutics (LOVIT), a newly established joint research unit between the Luxembourg Institute of Health and DKFZ. His team focuses on the development of new anticancer strategies based on oncolytic autonomous parvoviruses (PVs) with the ultimate goal to move them into the clinic. Major areas of research are: i) generation of innovative oncolytic vectors: retargeted PVs, chimeric vectors of adenovirus-PV genomes, PVs expressing sh/miRNAs; ii) design and assessment of novel combination therapies using PVs together with other anticancer agents such as apoptosis inducers, immune checkpoint blockade and/or epigenetic modulators of gene expression. He earned his PhD at the University of Heidelberg in 2001, working on the identification of new cellular proteins interacting with human papillomavirus 16 E7 oncoprotein. Before becoming Principal Investigator, he worked as a Postdoc Scientist at the University of Heidelberg on the characterization of the cellular functions of the SHOX homeodomain protein.

Q What is an oncolytic virus?

Oncolytic viruses are viruses that selectively infect, replicate in and kill cancer cells without harming normal tissues. Their life cycle ends with the lysis of the cancer cell and the release of new progeny viral particles into the tumor bed. These viral particles can then propagate in other cancer cells through a second round of amplification and cell lysis.

Importantly, virus-induced cell lysis is generally immunogenic and is often associated with the release of tumor-associated antigens. In the local

inflammatory tumor microenvironment that is established during oncolytic virus replication, these antigens alert and activate the immune system to act against the tumor. In this way, the immune system becomes the best ally of the virus in eliminating the cancer cells, even those not directly infected by the virus, such as cells forming small disseminated metastases.

Q How did the idea to use these viruses as tools to kill cancer cells emerge?

Oncolytic viral therapy is an old concept that has been around for more than a hundred years. The idea emerged when it was reported that some cancer patients who contracted influenza or other virus-related infectious diseases went into transient clinical remission. Despite early promise, however, the approach raised safety concerns because the viruses used as anti-cancer agents were human pathogens. With the advent of chemotherapy to treat cancer, oncolytic viral therapy was almost completely abandoned until the late 1980s.

The field has attracted renewed interest in recent decades, thanks to breakthroughs in genetic engineering and recombinant DNA technology that have enabled us to gradually increase our understanding of the virus life cycle and the factors involved in virus pathogenesis. This improved knowledge has made possible the development of new oncolytic viruses with excellent safety profiles and higher tolerability, specificity and potency.

As a result, numerous clinical trials have been launched in the past 20 years to evaluate at least 12 oncolytic viruses from 10 virus families. One of these viruses, Amgen's T-VEC, an engineered herpes simplex virus that contains the gene encoding the immunostimulatory cytokine GM-CSF, was approved by the US Food and Drug Administration in 2015 for the treatment of unresectable metastatic melanoma. This major breakthrough for the field has prompted justified optimism that other oncolytic viruses may soon be approved for other tumor types.

The immunostimulatory properties of oncolytic viruses – and their capacity to break tumor immune tolerance and convert a non-immunogenic tumor microenvironment into an immunogenic one – open up the possibility of combining oncolytic viruses with other forms of immunotherapy, such as immune-checkpoint blocking antibodies. Now that oncolytic viral therapy has reached new heights, let's hope that the enthusiasm in the field is translated into novel therapeutic options for cancer patients.

Q How safe is it to use oncolytic viruses as anti-cancer agents in the clinic?

Treatment with oncolytic viruses is considered extremely safe. To date, several thousand cancer patients have been treated with different oncolytic viruses in more than 100 clinical trials. These clinical studies have shown that the virus treatment is safe and has excellent tolerability. The most serious adverse side effects that have been documented so far are fatigue and temporary fever-like symptoms.

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Q Parvovirus is the latest addition to the oncolytic viral therapeutic tool kit. Can you tell us a little bit about the biology of parvovirus and the features that make this virus a potent effector against cancer?

Parvoviruses are single-stranded DNA viruses that are non-enveloped and have an icosahedral capsid of about 25 nm in diameter. Similar in size to a ribosomal subunit, parvoviruses are among the smallest viruses in nature; the name 'parvos' means 'small' in ancient Greek and Latin.

Although the parvoviruses that are currently under consideration as oncolytic viruses have rodents as their natural host, they can infect and kill human tumor cells. Several other features make these parvoviruses particularly attractive as anti-cancer agents. First, as rodent parvoviruses have never been associated with any human disease, there is no pathogenicity for humans. Also, because humans have not generally been exposed to rodent parvovirus infection, they lack pre-existing antiviral immunity – so the virus has more time to exert its oncolytic activity before the body forms antiviral antibodies to neutralize it.

> Rodent parvoviruses have never been associated with any disease in humans, and parvovirus treatment has been proven to have an excellent safety profile.

Second, these viruses have natural oncotropism – that is, they can specifically replicate in cancer cells by taking advantage of some of the genetic defects that distinguish cancer cells from normal cells, such as fast replication, defective signaling pathways and antiviral innate immunity. Third, rodent parvoviruses have multimodal oncolytic and onco-suppressive activities. Studies performed in a variety of cell culture and animal models have demonstrated that these viruses can induce different cell death processes, including apoptosis, necrosis and cathepsin-mediated cell death. Through these mechanisms, parvoviruses can efficiently kill many cancer cells, including cells that are resistant to other anti-cancer treatments. Importantly, parvovirus-induced cell death is often immunogenic, and parvoviruses can elicit robust anti-cancer immune responses. As a result of this immunostimulatory activity, the immune system plays a key role in parvovirus-mediated onco-suppression.

Finally, the small size of parvoviruses facilitates their spread in the tumor bed and allows them to cross physical barriers, such as the blood-brain barrier, that larger viruses cannot traverse.

Q Could you give us an update on the clinical trials being conducted using oncolytic parvoviruses?

Colleagues at the German Cancer Research Center, namely the Team headed by Jean Rommelaere, in collaboration with the University Medical Hospital of Heidelberg namely Karsten Geletneky

and the company Oryx have recently completed a clinical trial in patients with recurrent glioblastoma. The Phase 1/2a dose-escalation trial, which was the first clinical study in Germany to use oncolytic viruses, involved 18 patients in two groups. Oncolytic H-1 parvovirus (H-1PV) was given intratumorally to the first group and systemically to the second group. In both groups, patients had their tumor resected after 9 days and were injected with another half dose of the virus into the tumor resection cavity. The main end point of the trial was to evaluate the safety of the treatment and its tolerability.

The trial showed that H-1PV was safe and well tolerated. It also showed that the virus treatment was associated with initial signs of efficacy, such as the ability of the virus to cross the blood–brain barrier, wide spreading of the virus within the tumor bed, and evidence of virus-induced tumor necrosis. Furthermore, it was found that virus treatment correlated with immunogenic conversion of the tumor microenvironment, which was characterized by the infiltration of macrophages and active T-lymphocytes. This resulted in favorable progression-free survival and overall survival for patients in both groups in comparison with historical controls. These results are encouraging and make us very optimistic for the future.

At the moment, there is another Phase 1/2a trial using wild-type H-1PV underway in patients with pancreatic carcinomas.

Q What are some of the strategies being developed to increase the safety and efficacy of parvovirus therapies?

Rodent parvoviruses are not human pathogens and have never been associated with any disease in humans, and parvovirus treatment has been proven to have an excellent safety profile.

Unfortunately, parvovirus-based treatment has not eradicated tumors in patients. This calls for new developments to improve its clinical efficacy, which is the mission of my laboratory. To this end, we are currently focusing on three lines of research. First, we are designing new treatments that combine the virus with other anti-cancer agents. Second, we are constructing a second generation of parvoviruses with improved anti-cancer activity. And third, we aim to identify factors important for the virus life cycle that could serve as prognostic markers and help us to predict which cancer patients will most likely benefit from parvovirus-based treatments.

Q What are your thoughts on parvovirus-based combination therapies and what advantages do they offer compared to parvovirus alone?

We know that tumors are quite heterogeneous diseases; even within a single tumor, there can be a fraction of cancer cells that are weakly susceptible or even resistant to virus infection. These cells could be responsible for cancer relapse. One logical way to increase virus efficacy is to combine the virus with other anti-cancer drugs that use a complementary mechanism of action to target and kill infection-resistant

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cancer cells or to make them more susceptible to virus infection. Alternatively, we could use drugs that potentiate the anti-cancer activity of the virus, for example by increasing virus replication in cancer cells or improving the capacity of the virus to induce cancer cell death or elicit anti-cancer immune responses.

Combination therapy is one of the main research activities we pursue in my lab, and we are investing a lot of time and effort into identifying drugs that could synergize with parvovirus in killing cancer cells.

Q Can you tell us more about the next generation of parvovirus vectors you're working on in your laboratory?

Researchers working with other oncolytic viruses have been successful in potentiating their anti-cancer activity by inserting a therapeutic transgene into the viral genome. This is challenging for parvoviruses, however, because they have a very limited packaging capacity and can accept only transgenes with a maximum size of 200–250 bases. This greatly limits our ability to arm parvoviruses with large transgenes.

In my laboratory, we have successfully inserted a short hairpin RNA (shRNA) expression cassette into the parvovirus genome. This virus, which we call H-1PV silencer, expresses shRNAs at high levels and is very efficient in gene silencing, while retaining its ability to replicate. In an unpublished proof-of-concept study using both cell culture and animal models, we demonstrated that by expressing shRNAs against a certain oncogene, the virus acquired greater anti-cancer activity than the wild-type virus used in the glioblastoma clinical trial. Our work opens up the possibility of further exploiting H-1PV silencer to silence other genes involved in carcinogenesis or tumor development.

Another promising approach is the development of innovative chimeric viruses. These genetically engineered viruses contain elements of different viruses. We have constructed a first generation of chimeras, so-called adenovirus-parvovirus chimeras, by inserting a genetically engineered version of the H-1PV parvovirus genome into the genome of an adenovirus.

In the proof-of-concept study, we showed that an adenovirus containing the hybrid adenovirus-parvovirus genome efficiently infected cancer cells and produced full infectious parvovirus particles. Like a Trojan horse, the adenovirus is used as a shuttle to carry the parvovirus genome into the cancer cells. The parvovirus particles maintain their ability to infect and kill cancer cells and to induce secondary rounds of infection, thus amplifying the chimera's efficacy. Within this hybrid vector, we can insert larger transgenes into the adenovirus component of the genome and thus circumvent the small packaging capacity of the parvovirus. Essentially, we are combining oncolytic virus therapy with cancer gene therapy. We are currently working hard to develop this new technology and to investigate the therapeutic potential of adenovirus-parvovirus chimeras, alone and in combination with other anti-cancer agents. If we can provide preclinical evidence of their superior anti-cancer activity, we will pave the way for their use in cancer patients.

Q What are some of the key challenges that remain for the successful translation of parvovirus-based anti-cancer therapies to the clinic?

One of the greatest challenges in cancer research is the development of more relevant and reliable cancer models for proof-of-concept validations of anti-cancer agents. We need models that can better predict the clinical success of a new therapy. This is really evident if we consider that many of the anti-cancer agents that successfully arrested tumor growth or even eradicated tumors in rodent models have failed to provide significant benefits to patients once transferred to the clinic.

The major challenge for us, working with parvovirus at the preclinical level, is the need to improve efficacy. We believe that two areas of research may hold the key to the success of parvovirus in clinical settings: the identification of drugs that could synergize with the virus in killing cancer cells and the development of a second generation of viruses with enhanced anti-cancer activity.

I also believe that we must continue basic research to characterize the parvovirus life cycle and identify the cellular factors that play an essential role in modulating virus replication and killing activity. Such parvovirus modulators could not only serve as prognostic markers for patient stratification, but also guide the development of novel parvovirus-based combination strategies.

Based on the promising findings we have obtained thus far, we plan to multiply our efforts to improve parvovirus anti-cancer efficacy and translate our results into new cancer therapeutics. Our mission is to ensure that our research activities make a difference for cancer patients.

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