Abstract

Oncolytic viruses are replication competent “live” viruses. They infect tumor cells, replicate highly selective inside and thereby destroy them. Because of the enormous advances in the field of genetic engineering and biotechnology during the last decade, virotherapy is increasingly used within clinical trials and proved to be safe and effective. In particular, treatment of ovarian cancer patients is one main focus of research. On the other hand, this is due to the poor prognosis of this dismal entity, resulting in the urgent need for novel therapeutic strategies. From the other hand, as ovarian cancer typically spreads within the peritoneal cavity, intraperitoneal administration of oncolytic viruses is feasible. This paper provides an overview of promising results from clinical trials to treat ovarian cancer patients with oncolytic viruses.

Introduction

Every year, around 9600 women in Germany develop ovarian cancer. This makes it the fifth most common type of cancer in women. Because of its rare symptoms, 65% of the cases are diagnosed at a very late stage (FIGO III–IV) [1]. Despite advanced surgical techniques and modern systemic therapies (chemotherapy, targeted biological therapies), the 5-year probability of survival (around 30%) has barely improved at all over recent decades [2,3]. New therapeutic approaches are therefore urgently needed. The treatment of ovarian cancers using oncolytic viruses offers a very promising approach [4]. These are “living” agents which specifically infect and kill tumour cells as part of the virus replication process. Huge numbers of progeny virions are released, which in turn attack further tumour cells. The capability of constant, tumour-specific replication is a property that sets virotherapy apart from classical gene therapy, in which viral vectors that are not able to replicate are used to insert foreign genetic material into cells. Moreover, oncolytic viruses can also be used as “gene carriers” to enhance their antineoplastic effects. In contrast to classic gene therapy, the therapeutic transgene, coupled with the viral vector from which it is coded, spreads out within the tumour. This overcomes the hitherto primary transduction inefficiency of tumour cells, a significant limitation in gene therapy for cancer [5]. The use of oncolytic viruses to treat tumours is not a new idea. Interestingly, viruses with natural
Oncolytic viruses that have been used in clinical phase 1 studies on the treatment of patients with ovarian cancer.

In normal cells but which neoplastic cells can do without, and (3) tumour cells, (2) disabling a gene needed for efficient replication by (1) modifying the viral envelope to allow selective uptake into the tumour. This is achieved by a number of mechanisms, including a deletion of genes that are not needed in neoplastic cells, or the introduction of genes that are not present in normal cells. This is one of the reasons why various viruses prefer to grow in tumour cells. Viruses with natural oncolytic properties include Newcastle Disease viruses (NDV) [12], Vaccinia viruses VV [13], vesicular stomatitis viruses (VSV) [14], parvovirus H1 (H-1PV) [15], measles vaccine viruses (MeV) [16] and reoviruses (RV) [17]. Viruses can also be genetically engineered so that they are dependent on neoplastic host cells to reproduce. The basic principles of virotherapy and its particular characteristics are also explained. Future challenges and the potential that oncolytic viruses offer will then be discussed.

Mechanisms of Tumour Selectivity

Throughout evolution, viruses have excelled at specialising in penetrating host cells and appropriating their biosynthetic apparatus. Thereby, they manipulate essential cell functions such as cell division, differentiation and cell death. These cellular changes are frequently very similar to the changes that a cell experiences during carcinogenesis (e.g. inactivation of the tumour suppressor gene p53, manipulation of the interferon system, stimulation of the cell cycle, suppression of apoptosis) [12]. This is one of the reasons why various viruses prefer to grow in tumour cells. Viruses with natural oncolytic properties include Newcastle Disease viruses (NDV) [13], Vaccinia viruses VV [14], vesicular stomatitis viruses (VSV) [15], parvovirus H1 (H-1PV) [16], measles vaccine viruses (MeV) [17] and reoviruses (RV) [18]. Viruses can also be genetically engineered so that they are dependent on neoplastic host cells to reproduce. This is achieved by (1) modifying the viral envelope to allow selective uptake into tumour cells, (2) disabling a gene needed for efficient replication in normal cells but which neoplastic cells can do without, and (3) creating tumour or tissue-specific promoters that regulate the expression of viral genes [12]. It is also possible to combine these approaches [19]. Table 1 provides an overview of oncolytic viruses that are already used in clinical studies to treat patients with ovarian cancer.

<table>
<thead>
<tr>
<th>Virus Name</th>
<th>Mechanism of tumour selectivity</th>
<th>Result</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measles vaccine virus</td>
<td>Natural tumour selectivity</td>
<td>Good tolerance. Dose-dependent stabilisation of the progress of the disease in 14 out of 21 patients with an average duration of 93 days.</td>
<td>[24]</td>
</tr>
<tr>
<td>Onyx-015</td>
<td>Deletion in the E1B and E3B gene (tumour selectivity for cells with defective p53 signal transduction pathway and defective RNA transport)</td>
<td>Good tolerance. No clear radiological or clinical tumour response.</td>
<td>[39]</td>
</tr>
<tr>
<td>H101</td>
<td>Deletion in the E1B and E3B gene (tumour selectivity for cells with defective p53 signal transduction pathway and defective RNA transport)</td>
<td>Good tolerance. 3/9 patients with complete remission, 2/9 with partial remission and 4/9 with no tumour response.</td>
<td>[40]</td>
</tr>
<tr>
<td>Ad5-delta24-RGD</td>
<td>Binds to αvβ3 and αvβ5 integrins; deletion in the E1A gene (tumour selectivity for cells with defective retinoblastoma protein-dependent cell cycle control)</td>
<td>Good tolerance. 15/21 patients with stable disease, 6/21 with progressive disease and 7/21 with decreasing CA125.</td>
<td>[42]</td>
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</tbody>
</table>

Viruses with Natural Tumour Selectivity

Living viruses capable of replication have already been used millions of times in the context of vaccination and are known to be extremely safe therapeutic agents with low side effects [20]. The use of “live” vaccine viruses for oncolytic virotherapy therefore would seem to be an elegant approach. Interestingly, some vaccine strains replicate better in neoplastic cells than the corresponding wild type viruses. Measles vaccine viruses, for example, have natural oncolytic properties. In contrast to wild type measles virus they predominately enter cells via the CD46 receptor which is over-expressed by malignant cells including ovarian cancer [21,22]. An innovative approach was described by Peng et al at the Mayo Clinic in Rochester, USA: they generated a measles vaccine virus encoding for the human carcino-embryonic antigen (CEA) (MeV-CEA) [23]. During virotherapy with MeV-CEA, a simple blood test can be taken to determine the CEA level, thereby allowing viral replication to be monitored in real time. Galanis et al. recently published the results of a phase I trial on the intraperitoneal use of MeV-CEA in patients with advanced ovarian cancer [24]. The virus application was well tolerated, could easily be monitored by determining serum CEA levels and demonstrated promising clinical activity.

Another vaccine virus, the Vaccinia virus (VV), has successfully been used to treat smallpox. Numerous clinical studies have also demonstrated that VV has natural oncolytic properties [25–28]. The use of VV for oncolytic virotherapy is regarded as very safe and generally only causes mild, flu-like symptoms. Disabling two viral genes enhances tumor selectivity: thymidine kinase (TK) enables the virus to replicate independently of the host cell’s cell cycle, and the Vaccinia growth factor (VGF, similar to the epithelial growth factor EGF) makes it easier for the virus to infect neighbouring cells [29]. Both TK and EGF are over-expressed by many tumour cells, which is why their deletion within the VV genome makes virus replication more difficult in non-neoplastic cells, while neoplastic cells are able to produce large volumes of progeny viruses. Pre-clinical studies using VV to treat ovarian cancer demonstrated an excellent anti-tumour activity [30]. In view of the large virus genome, VV is also an excellent vector for
additional therapeutic transgenes. Chalikonda et al. generated a
VV encoding for the suicide gene cytosine deaminase (CD) (vvDD-CD). This converts the non-prodrug prodrug 5-FC into cyto-
toxic 5-FU. In an animal model to treat ovarian cancer, the addi-
tion of the prodrug increased the oncolytic activity of vVD-CD in
a tumour-specific and highly significant manner [31].
Multiple phase I/II clinical trials using VV are currently being
carried out on the treatment of ovarian cancer (http://www.
jenerex.com). Currently, the first german virotherapy phase I trial
to treat therapy resistant peritoneal carcinosarcoma is initiated, which
includes a large proportion of ovarian cancer patients with
peritoneal recurrence (http://www.clinicaltrials.gov/ct2/show/
NCT01443260;term=GL-ONC1&ra=1).
One of the first virotherapy approaches for the treatment of ova-
rian cancer was the use of oncolytic reoviruses. These double-
stranded RNA viruses replicate highly selectively in tumour cells
with an activated Ras signal transduction pathway. Hirasawa et
al. demonstrated in animal models that reoviruses are able to
shrink ovarian cancer, reduce the formation of ascites and signifi-
cantly prolong the survival of animals given this treatment [32].
Reoviruses of serotype 3 (Reolysin®, Oncolytics Biotech) are
currently being used in numerous clinical phase I and II trials
that include to treat advanced ovarian cancer (http://www.
clinicaltrials.gov/ct2/results?term=Reolysin) [33]. Following both,
intrapерitoneal and intravenous virus application, there was ex-
cellent tolerance, tumour-specific viral replication and oncolytic
activity [34,35].

Viruses with Genetically-Engineered
Tumour Selectivity

In many cases, viral gene products require the proliferation of the
host cell or inhibit anti-viral defence mechanisms. Since tumour
cells proliferate actively and frequently have limited viral de-
defences, the disabling of certain viral genes brings about artificial
tumour selectivity. Consequently, the adenoviral protein E1B
binds to and inactivates tumour suppressor p53, thereby promot-
ing continuous viral replication [36]. Disabling E1B accordingly
leads to the targeted infection of cells with defective p53 signal
transduction pathway. Both adenoviruses Onyx-015 and H101
(Sunway Biotech, Shanghai, China) have corresponding deletions
in the E1B gene [37,38]. Onyx-015 was the first genetically modi-
fied oncolytic virus to be used in clinical studies. Although the
virus demonstrated promising oncolytic activities in pre-clinical
studies, a phase I trial on the treatment of patients with ovarian
carcinoma showed no clear clinical or radiological tumour re-
sponse [39]. H101 is the first oncolytic virus to receive market
approval (in China, not in western countries) based on phase III
trials. A phase I trial on the treatment of malignant ascites in
ovarian cancer patients led to a significant reduction in the fre-
quency of paracentesis, which markedly improves quality of life
[40].
The primary point of attack for the adenoviruses mentioned is the
Coxsackie adenovirus receptor (CAR). The reason for the in-
adequate clinical effectiveness of Onyx-015 in the treatment of
ovarian cancer may be the highly variable expression of CAR and
a resulting inadequate transduction efficiency of the addressed
tumour cells. Genetic modifications of the viral envelope may ac-
cordingly lead to an increased binding affinity towards ovarian
cancer cells. The adenovirus Ad-delta24-RGD, for example, binds
to integrins in the cell surface, including those of ovarian carcino-
ma cells [41]. The adenoviral E1A protein also lacks the binding
point for the cell cycle-regulating retinoblastoma (Rb) protein.
Consequently, Ad-delta24-RGD replicates selectively in cells with
an inactive Rb signal transduction path and accordingly in many
neoplastic cells, including ovarian carcinomas. In a phase I trial
on the treatment of patients with gynaecological cancers, the in-
terperitoneal administration of Ad-delta24-RGD was well toler-
ated [42]. Replication of Ad-delta24-RGD in the patients’ ascites
and promising clinical activity was also demonstrated.

Challenges and Requirements of Oncolytic Therapy

Genetic stability is important both for production technology and
safety-related reasons. Ultimately, it must be possible to produce
the virus easily and efficiently (i.e. with a high titre). Vaccine vi-
ruses in particular (live vaccines) satisfy these requirements. In
light of the many years’ experience involving enormous patient
numbers, there is plenty of experience available regarding safety
and side effects. Technology is available for efficient virus produc-
tion with high quality requirements of the production processes,
which also contribute towards a high degree of genetic stability.
One disadvantage of using vaccine viruses, however, is the high
seroprevalence for the agent. With systemic application in partic-
ular, which appears to be the medium of choice for advanced can-
cer, oncolytic viruses are not only subjected to the innate im-
une response, but also to acquired defence mechanisms [43].
When treating ovarian cancer, the frequent loco-regional disease
spread lends itself to intraperitoneal application. Although anti-
viral antibodies may be present in malignant ascites, a phase I
study shows that the intraperitoneal use of measles vaccine
viruses does not cause a rise in the antibody titre and that the tu-
mour response does not correlate with the pre-therapeutic pres-
ence of anti-measles antibodies [24,44]. Various approaches to
counteract anti-viral immune responses have also been de-
scribed. On the one hand, there are approaches which eliminate
viruses by modulating the immune response, for example
through the simultaneous application of immuno-suppressive
substances [45,46]. Another approach is one taken naturally by
many viruses: by infecting endogenous, circulating cells, they
mask themselves from the immune system. In an analogy to this,
oncolytic viruses can be administered in carrier cells and deliv-
ered to the primary tumour concealed (“Trojan Horses”) [47].
This will ensure that the agent is no longer recognised by the
immune system. Viral replication can also take place within the
“Trojan”, and the carrier cells can contribute towards the tumour
selectivity by selecting cells with inherent tumour tropism [48].
The consequences of the immune response, however, do not all
have a negative effect on the effectiveness of virotherapy. The in-
teraction of the immune system with virus-infected cells appears
to contribute to the oncolytic activity in vivo and in particular in-
duce positive long-term effects by stimulating the anti-tumour
immune defence. These effects can be amplified by cloning trans-
genic immuno-modulators into the viral genome. The problem
when investigating interactions of oncolytic viruses with the im-
une system, however, is that immune-compromised Xenograft
mice are frequently used as the tumour model. Extensive trans-
slational research in the context of clinical trials to characterize
immuno-virotherapeutic effects is therefore essential and is one
main interest of the German Consortium for Translational Cancer
Research (DKTK), which is currently being set up. In an innova-
tive clinical approach led by A. Hemminki (Advanced Therapy

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Access Program), patients with advanced, solid tumours that are refractive to treatment (including patients with ovarian cancer) are treated with adenoviruses that express GMCSF (Granulocyte Macrophage Colony Stimulating Factor) [49,50]. GMCSF stimulates the anti-tumour immune response by activating CD8+ T lymphocytes and natural killer cells. The treatment is tolerated well and has positive effects in the majority of the patients treated. There is also an anti-tumour as well as an anti-viral immune response. This in particular indicates that the immunological tolerance to tumour tissue can be broken through by oncolytic viruses.

**Summary and Outlook**

Virotherapy is a highly promising approach to treat ovarian cancer. Several clinical trials have demonstrated the therapy’s clinical effectiveness. Unlike intraperitoneally administered chemotherapy, intraperitoneal virus administration is tolerated very well [51]. The-wholly different method of action compared to that of classic cytostatics meaning on the one hand that tumours resistant to chemotherapy could be sensitive to oncolytic viruses [52, 53]. On the other hand, the occurrence of negative side effects is not anticipated when combined treatment involving oncolytic viruses and classical forms of treatment is given. Oncolytic viruses are also of interest as a vehicle for therapeutic transgenes in relation to a whole variety of genetic therapy constructs. As well as generating oncolytic viruses that are optimised for the treatment of ovarian cancer, future studies should also analyse the ideal form of virus administration, the identification of potential therapeutic combination partners and the interaction of virotherapy with the immune system of affected patients.

**Conflict of Interest**

None.

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