Oncolytic Viruses as a Possible Therapeutic Strategy against Malignant Pleural Mesothelioma

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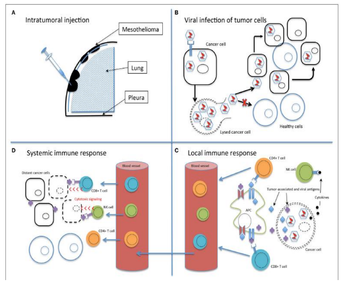
Introduction and Aim

Malignant pleural mesothelioma (MPM) is a very aggressive cancer correlated to asbestos exposure. MPM is resistant to the current therapeutic strategies and despite multi modality treatments its prognosis remains dismal, with a median survival of 9-17 months from diagnosis [1]. As the incidence of MPM is predicted to increase in the next decade [2], the identification of new effective therapeutic approaches is an urgent need.

Cancer therapy through oncolytic viruses (OVs) has always been considered a promising therapeutic approach that have recently found a successful application in the clinical setting [3]. MPM represents an ideal candidate for virotherapy for numerous reasons including the frequently localized pattern of growth and the pleural location, which allows direct access for the intra-tumoral injection of the Ovs [4].

We tested the potential antitumor effects of two the oncolytic strategies: 1) the adenovirus *dl922-947* in both MPM cell lines and mouse xenografts and 2) the effects of a non-human Caprine Herpesvirus-1 (CpHV-1) on a panel of MPM cell lines both alone and in combination with cisplatin. Our data suggest that these Ovs, alone or in combination, could be a feasible strategy against MPM.



Results

Analysis of *dl*922-947 effects on MPM cell lines

We focused on adenoviruses with a 24 bp deletion in the E1A-conserved region 2, which binds and inactivates the retinoblastoma protein, resulting in a virus (***dl*922-947**) [5] that cannot trigger S phase entry in normal cells, but can still replicate in cells with an aberrant G1-S checkpoint, a defect observed in over 90% of human cancers, including MPM (Figure 1). First, we assessed the effects of the virus on a panel of MPM representative of the main different histotypes: the epithelioid NCI-H28 and NCI-H2452, the biphasic MSTO-211H and the sarcomatoid NCI-H2052. By sulforhodamine B (SRB) assay we determined the IC50 values at 5 days following treatment with *dl922-947* and found that all MPM cell lines were susceptible to viral treatment (Table 1), except NCI-H2052 cells, in which viral entry was not efficient, as shown through infection with a reporter adenovirus transducing GFP (not shown).

Figure 1

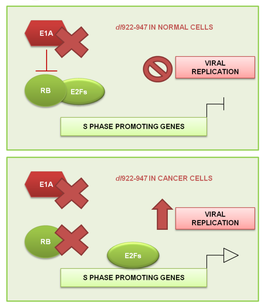


Table 1

cell line	IC50 (pfu/cell) *dl*922-947
NCI-H28	4.3
MSTO-211H	5.3
NCI-H2452	103.6
NCI-H2052	undetermined

*dl*922-947 induces apoptosis and hyperdiploidy in MPM cell lines

We then analyzed by cytofluorimetric analysis the effect of *dl922-947* on cell cycle features of responsive cells and we found that *dl922-947* treatment at the IC50 and IC50/2 induces an increase of the subG1 cell fraction, suggestive of cell death, and of the hyperdiploid (4N) population, suggestive of mitotic defects. The *dl922-947* treatment also induced the expected S phase increase in NCI-H2452 and NCI-H28 but not a significant increase in MSTO-211H at the doses and times analyzed. Induction of cell death was further confirmed in NCI-H28 and MSTO-211H through annexin V assay (**Table 2**).

Table 2

		Apoptosis (%) Early	Late	Total	
H28		11.87	3.05	14.92	Control
		41.50	12.06	53.56	*dl*922-947
MSTO		1.60	0.70	2.30	Control
		50.83	16.79	67.62	dl922-947

Testing dl922-947 in combination strategies

Oncolytic adenoviruses can interact synergistically with different drugs [6]. In particular, we tested *dl*922-947 efficacy in combination with cisplatin that is used as first-line treatment for MPM and in combination MK-1775, a first-in-class inhibitor of the WEE1 kinase, which we previously found able to sensitize MPM cells to cisplatin [7] and which is currently being tested in clinical trials for different tumor types. By analyzing different schedules of treatment, we found that both cisplatin treatment MK-1775 are able to increase the cytotoxic effect of the oncolytic virus treatment upon 24h from *dl*922-947 infection (**Figure 2**).

dl922-947 inhibits tumor growth in vivo in a xenograft model of MPM

MPM xenografts whereby athymic mice were inoculated subcutaneously with MSTO-211H cells and when the tumorsbecame palpable, the mice were divided into two groups (of 9 animals each) and treated bi-weekly with *dl922-947*. Virotherapy proved extremely effective in counteracting tumor growth as early as after 3 weeks of treatment: the animal treated showed total tumor regression already after the first week, and other two mice had similar results during the course of treatment. Animals that had shown total tumor regression were not sacrificed at the end of the experiment and were observed for 3 additional months. During this period, no tumor re-growth was observed **(Figure 3).**

Figure 2

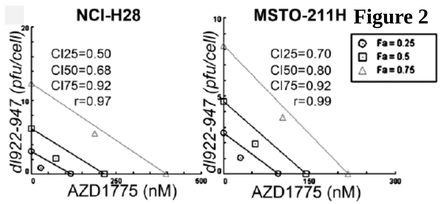
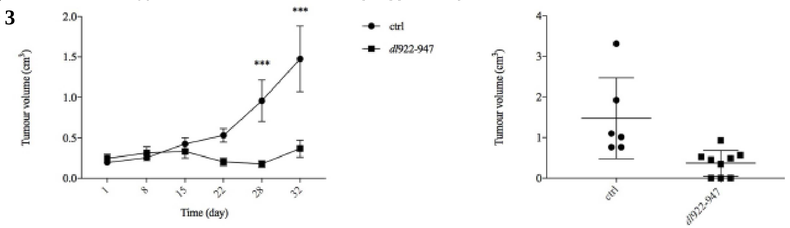


Figure 3



Oncolytic Caprine Herpesvirus 1 (Cphv-1) induces apoptosis and synergizes with cisplatin in MPM cell lines

Among the different types of OVs now available, some non-human wild-type OVs show advantages over human OVs, including the inability to replicate in normal human cells while having a natural tropism for human cancer cells and the absence of pre-existing immunity [8], so we also assessed CpHV-1 effects on MPM (NCI-H28, MSTO, NCI-H2052) and non-tumor mesothelial (MET-5A) cells. We found that CpHV-1 reduced cell viability and clonogenic potential in all MPM cell lines without affecting non-tumor cells, in which, indeed, we did not detect intracellular viral DNA after treatment. In particular, CpHV-1 induced MPM cell apoptosis (**Figure 4B**) and accumulation in G0/G1 or S cell cycle phases. Moreover, CpHV-1 strongly synergized with cisplatin and this agent combination did not affect normal mesothelial cells **(Figure 4A).**

Figure 4B

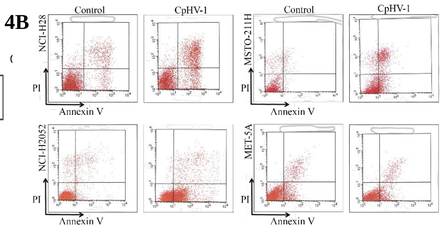
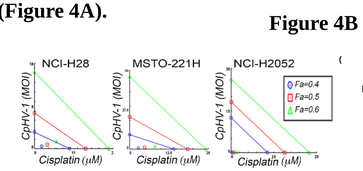


Figure 4A



Conclusion

Our preliminary data on the potential use of virotherapy based on the *dl922-947* oncolytic virus and on CpHV-1 virus suggest that OV could be an effective strategy for the treatment of MPM. We plan to further carry on the preclinical characterization of OVs use both as single agent and in combination strategies and we will analyze the molecular mechanisms underlying apoptosis induction and whether viral entry into the cells and viral replication are affected by combinatorial approaches. We will then assess whether this strategy is feasible also on orthotopic MM models.

References

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