Potentiating effect of reovirus in anti-PD1 therapy in colorectal cancer

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Session: PO.ET07.03

Poster # 3917

ABSTRACT

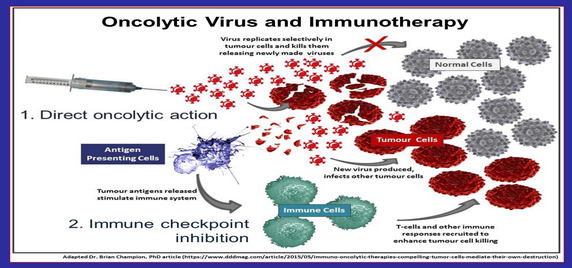
Background:
Microsatellite instability (MSI) high colorectal cancers (CRCs) have deficiency in mismatch repair (MMR) and increased levels of PD-L1, LAG-3, and IDO and respond positively to anti-programmed death (PD) therapy. MSI low or microsatellite stable (MSS) CRCs that make up majority of tumors in clinical practice have not seen any benefit with PD inhibition. MSS CRC has higher proportion of *KRAS* oncogenic mutations as compared to MSI CRC. Reovirus, a naturally occurring oncolytic double-stranded RNA virus, has intrinsic preference for replication in *KRAS* mutant cells causing apoptosis in CRC. Current study was designed to investigate if reovirus could potentiate a beneficial effect of anti-PD therapy in MSS CRC.

Methods:
An array of CRC cell lines were screened for sensitivity to reovirus by MTT assay and expression of stem cell markers by RNA-Seq and FACS. Based on MSI and *KRAS* status, four cell lines were explored further. Cells were treated with 5MOI reovirus for 48hr and expression of PD-L1 and PD-L2 with and without the potentiating effect of IFN-γ was assayed using FACS and qPCR. Combinatorial effect of reovirus with anti-PD-1 agent was studied in syngeneic models of BALB/c (CT26; $KRAS^{mut}$, MSS) and C57BL/6 (MC38; $KRAS^{wt}$, MSI) mice. The mice were grouped as control (PBS/IgG_2A isotype control), reovirus, anti-mouse PD-1 antibody, and combination. Reovirus was used at a dose of 10 million/100 uL daily and anti PD-1 antibody was given i.p 200 ug/100 uL twice a week. Survival data and tumor volumes were recorded. At the end point, immunohistochemistry was performed with CD8 and granzyme B antibodies on excised paraffin fixed CT26 tumor tissue from BALB/c animals.

Results:
HCT116 (MSI, $KRAS^{mut}$), SW620 (MSS, $KRAS^{mut}$), LIM2405 (MSI, $KRAS^{wt}$) and HT29 (MSS, $KRAS^{wt}$) were chosen based on the increased sensitivity to reovirus and expression of CD133, CD44 and CD24. HCT116, LIM2405 and SW837 revealed increased and HT29 reduced expression of PD-L1 upon treating with reovirus. Survival data and tumor volume measurements showed better potentiating effect of reovirus on anti-PD-1 therapy in CT26 syngeneic model when compared with MC38. While single agent therapy did not increase survival, the combination did improve survival with significance, vs control in both BALB/c (median 42 vs 16 days, p=0.003) and C57BL/6 (median 24 vs 17 days, p=0.02). The reovirus and anti PD-1 treated syngeneic model tumor tissue showed a higher infiltration of T lymphocytes as confirmed by CD8 positive and intensified granzyme B staining.

Conclusion:
Reovirus as single agent is more potent in $KRAS^{mut}$ CRC. Syngeneic mice models proved synergistic anticancer effect of reovirus and anti-PD1 agent combination. Reovirus administration increased PD-L1 expression in CRC cells, possible mechanistic rationale for synergistic efficacy.

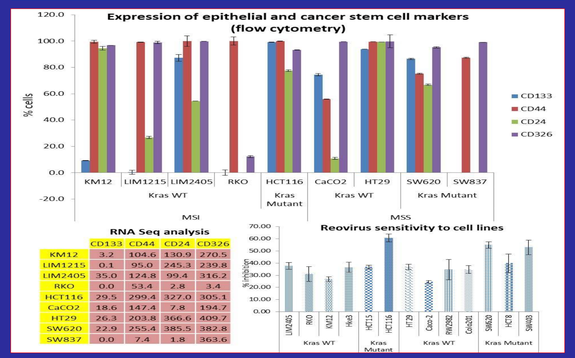
BACKGROUND & HYPOTHESIS



MATERIALS & METHODS

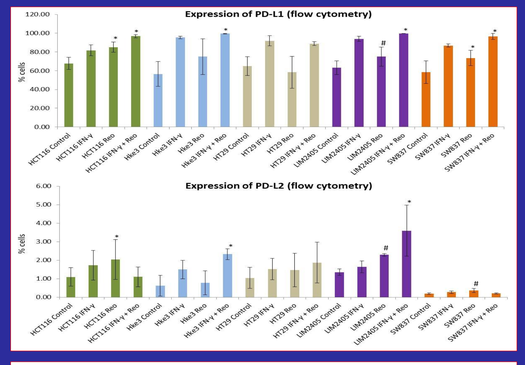
- **Human and mouse CRC cell lines were obtained from ATCC and as generous contribution from collaborators. All *in vivo* protocols were approved by the institutional animal care and use committee**
- **Reovirus obtained from Oncolytics Inc. as 2.53 X 10^{10} $TCID_{50}$ solution, IFN-γ treated as 1ng/mL after 24hr of cell seeding**
- **Anti-mouse PD1 antibody and IgG_2A isotype control were obtained from Leinco Technologies. Anti-CD8 and –granzyme B were obtained from antibodies-online Inc. and abcam**
- **Reovirus treatment for MTT assay and stem cell marker analysis (FACS) was 2.5 MOI for 48 hr. RNA-Seq experiments were performed in collaborator's laboratory**
- **Expression of PD-L1, PD-L2 and immune response mediators in cell lines were assessed by FACS and qPCR**

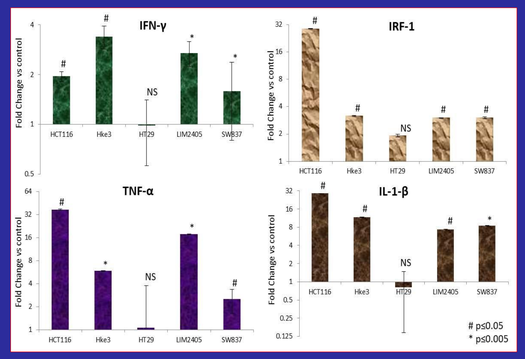
RESULTS

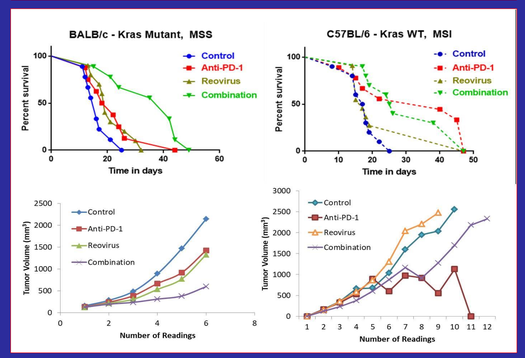


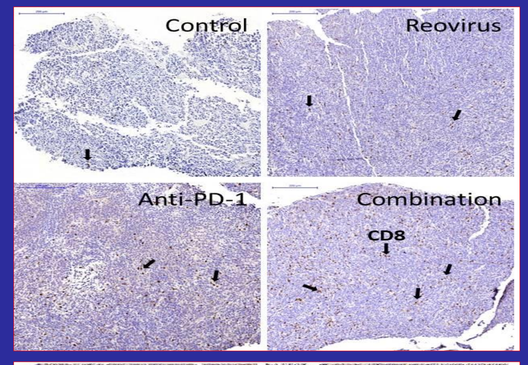
	CD133	CD44	CD24	CD326
KM12	3.2	104.6	130.9	270.5
LIM1215	0.1	95.0	245.3	239.8
LIM2405	35.0	124.8	99.4	316.2
RKO	0.0	53.4	2.8	3.4
HCT116	29.5	299.4	327.0	305.1
CaCO2	18.6	147.4	7.8	194.7
HT29	26.3	203.8	366.6	409.7
SW620	22.9	255.4	385.5	382.8
SW837	0.0	7.4	1.8	363.6

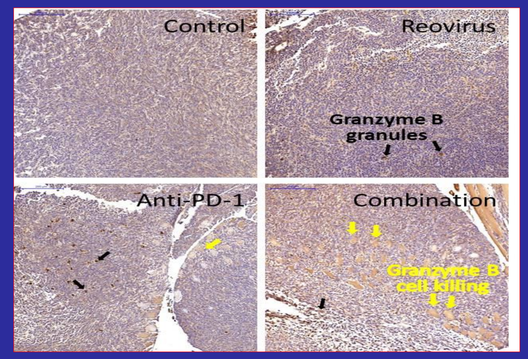
RESULTS & CONCLUSIONS











Conclusions

- **Administration of reovirus proved to be more cytotoxic in presence of activating mutations of *KRAS*, and increases the expression of PD-L1 and PD-L2 and immune response mediators such as IFN-γ, IRF-1, TNF-α and IL-1-β in CRC cells**
- **Combinatorial effect of reovirus with anti-PD1 therapy was demonstrated in syngeneic animal models - *KRAS* Mutant + MSS tumor-bearing mice had better survival and reduction in tumor volume compared to *KRAS* WT + MSI mice. Increased presence and cytotoxic activity of CD8-positive T cells observed upon treatment with reovirus and anti-PD1 antibody in BALB/c animals**
- **In-depth analysis and possible mechanism of action of this combination are underway**