Oncolytic Vaccinia virus contains a potent CD80/CD86 ligand whose deletion confers higher tolerance, and potential synergy with immune amrasing

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ABSTRACT

Vaccinia virus (VACV) has been intensively used as oncolytic virus for the treatment of various types of cancers over the last years. Targeted gene deletions have enabled the selection of new VACV variants that retain tumor-specific replication, and oncogenic properties, and are safer for the surrounding healthy tissues. Recent examples of such deleted VACVs are TG6002, J2LRRT (hM/LR-R) -FucI (Foloppe et al. 2019), or J2LRRT-J-L-FucI (Pain et al. 2019). It is also well established that VACV secretes various factors interfering with major immune pathways, largely contributing to immune evasion for the virus. However, to our knowledge, none of these factors has already been used in the field of cancer immunotherapy. We here report the identification of M2L as a strong binder to both CD80, and CD86 co-stimulatory receptors. This binding antagonizes the interaction with their natural ligands CD28, and CTLA-4. M2L can also stabilize the interaction between CD80, and PD-L1 in vitro. We characterized M2L as a secreted homo-oligomeric protein (8 × 35 kDa) and could determine that apparent affinities are in the same range as natural ligands. It proved as active as CTLA-4, as an inhibitor for lymphocyte activation in a MLR assay. The unique properties of M2L make it a potential new immuno-suppressive drug. Interestingly, M2L is largely conserved within the poxvirus family, and we could demonstrate that its ortholog from myxomavirus can also interact with CD86. Exposing to recombine the immunogenic properties of VACV, we engineered a triple-deleted Vaccinia virus (TD, Δ2LRrtΔM/LRΔM2LΔM2L). Oncolytic activity was not affected by the deletion of M2L, as assessed both in the regular mouse tumor xenograft models (HCT116), or in the syngeneic models (B16F10). We could demonstrate a better tolerance for the TD variant in a humanized model, where the CD80/CD86 pathway might be prominent in the neutralizing response. Finally, the TD oncolytic backbone might be interesting for the development of our invirIO™ platform.

RESULTS

1. M2L is a secreted homo-oligomeric protein

2. M2L binds to both CD80 and CD86

3. M2L protein inhibits interaction of CD80/CD86 with CD28/CTLA4, and favors binding of CD80 to PD-L1

4. VACV-TD (M2L-) lost binding to CD80/CD86

5. VACV-TD displays the same replicative and oncolytic activities as VACV-DD

6. Inhibition of lymphocyte activation (MLR assay) confirmed the function of M2L, and function loss in VACV-TD.

REFERENCES


CONCLUSIONS AND FUTURE STEPS

A new immunosuppressive function was disclosed for the M2L gene of vaccinia virus : patient applications have been filed for both M2L as an immunosuppressive protein, and for VACV-TD as an improved backbone for the development of armed oncolytic virus.

The functional characterization of M2L protein has been instated, and further studies need to confirm the role of M2L in tolerance VACV treatment, and the potential clinical utility of VACV-TD in the field of cancer immunotherapy.

M2L might also represent an attractive target in the field of auto-immune disorders or for treating complications of cancer therapies.

Transgene is open to any modality of collaboration for further characterization of the function of M2L in relevant translational models.

ACHIEVEMENTS

Identification of CD80 and CD86 as natural ligands of the M2L protein
- M2L prevents the interactions of CD80/CD86 to CD28 and CTLA4.
- M2L favors the interaction between CD80 and PD-L1
- M2L protein inhibits lymphocytes activation in MLR format
- VACV leads to M2L in vitro and in vivo oncolytic activity
- Deletion of M2L leads to better tolerated VACV

MAIN FEATURES OF ONCOLYTIC VACCINA

- Copenhagen strain: best oncolytic activity among VACV strains, and among orthopoxviruses
- Good safety profile and high therapeutic index; thymidin kinase (TK) and thymidine nucleotide reduction (RR) deletions restrict replication to proliferative cells (e.g. tumoral cells)
- Solid track record of clinical use (TG6002 currently in clinical trial, dose exc. up to 10^11 pfu/V)
- Large DNA insertions are possible (up to 25 kb), with successful vectorization of various expression cassettes (enzymes, cytokines, antibodies, etc.)
- Pure cytoplastic replication (no risk for genome integration or mutagenesis)
- Good immunological balance (anti-tumor vs anti-viral responses, Th1 vs Th2, etc.)
- Well established GMP manufacturing processes

REFERENCES