DR510: A Dual-Masking T-Cell Engager Prodrug with Single-Site Cleavage for Balancing Efficacy and Safety in Solid Tumor Therapy

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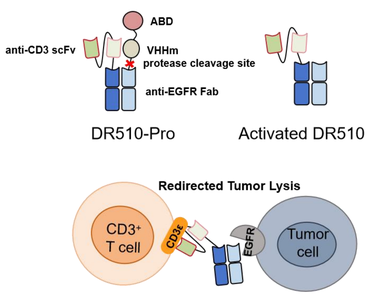
INTRODUCTION

Fig 1. Structure and MOA of DR510.



T-cell engagers (TCEs) face critical challenges in solid tumor treatment, including on-target off-tumor toxicities, cytokine release syndrome (CRS), and immune effector cell-associated neurotoxicity syndrome (ICANS). Tumor microenvironment-activated TCE prodrugs represent a promising strategy to overcome these dilemmas. Here, we report the development of DR510 (or DR510-Pro), an epidermal growth factor receptor (EGFR)-targeting TCE prodrug, leveraging a novel cleavable linker and a VHH masking domain (VHHm) blocking the SP34-derived anti-CD3 scFv. An albumin-binding domain (ABD) was fused to VHHm, which "locks" the moderate-affinity anti-EGFR Fab through interaction with the anti-CD3 scFv, achieving dual masking of both functional domains. Uniquely, DR510 features a single-site cleavage mechanism for ABD-VHHm release in circulation, distinguishing it from previously reported TCE prodrugs.

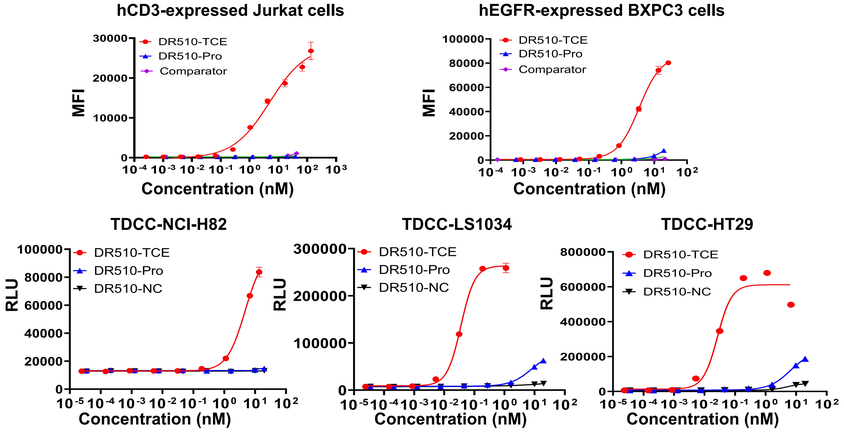
In vitro experiments showed significantly reduced hCD3/hEGFR binding and T-cell-dependent cytotoxicity (TDCC) effect of DR510 in its prodrug state, with functional restoration following enzymatic cleavage. DR510-Pro demonstrated superior tumor growth inhibition compared to a clinical-stage comparator at equimolar doses (0.066–0.2 mg/kg) across all models. In non-human primates, DR510-Pro exhibited favorable PK and robust tolerability at 0.65 and 1.3 mg/kg, with only minimal IL-6 elevation, indicating reduced CRS risk.

METHODS

- ☐ Binding interactions between DR510-Pro and hCD3/hEGFR were evaluated via flow cytometry.
- ☐ Enzymatic cleavage-induced T-cell activation was assessed in a TDCC reporter bioassay.
- ☐ *In vivo* efficacy was evaluated in human PBMC-engrafted CDX mouse models bearing HT29 or PC9 tumors and in a PDX mouse model bearing HNSCC.
- ☐ Pharmacokinetics (PK) and safety profiling were characterized in cynomolgus monkeys.
- ☐ Statistics were analyzed by one-way ANOVA using GraphPad Prism.

RESULTS

Fig 2. The decreased binding and T-cell activation ability of DR510.



Note: DR510-NC is constructed by replacing the cleavable linker with a uncleavable glycine-serine-rich linker

Fig 3. Functional activity of DR510 is cleavage-dependent.

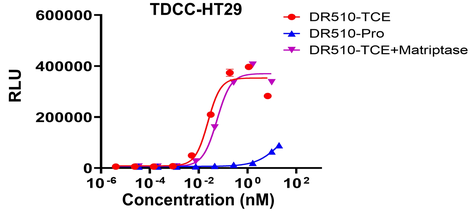
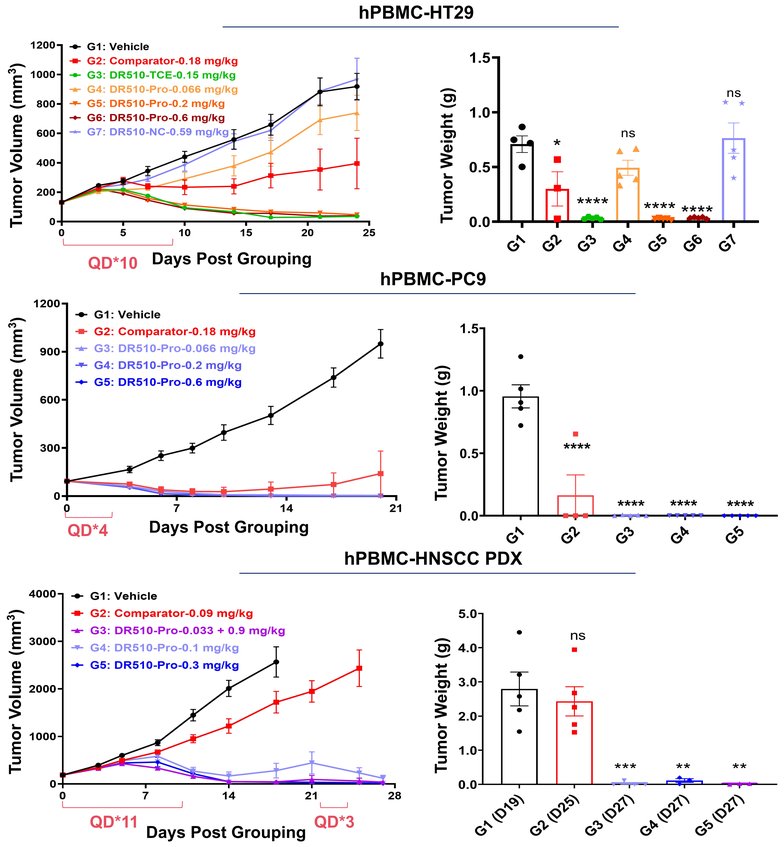
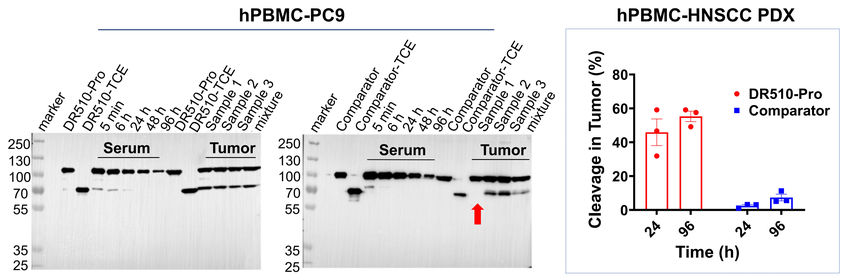


Fig 4. DR510 treatment induces tumor regression in a cleavage-dependent manner.



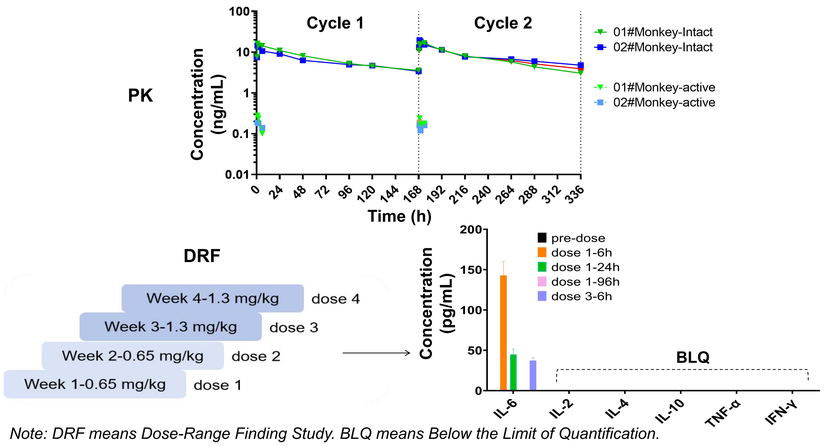
Note: Comparator is EGFR TCE prodrug with dual peptide masks.
*The data are shown as mean±SEM. ns: not significant, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 compared with G1: Vehicle group.*

Fig 5. DR510-Pro exhibits markedly superior cleavage efficiency within tumors.



Note: Mice bearing PC9 or HNSCC PDX tumors were each divided into 2 groups (n=3) and received a single tail-vein injection of 30 mg/kg DR510-Pro or an equimolar amount of the Comparator.
In one mouse bearing PC9 tumor from the Comparator group, no cleavage is observed (Sample 1, red arrow).

Fig 6. DR510-Pro shows favorable PK and excellent safety profiles in cynomulgus monkeys.



Note: DRF means Dose-Range Finding Study. BLQ means Below the Limit of Quantification.

SUMMARY&CONCLUSION

- ☐ **Effective prodrug masking:** The activity of DR510 is effectively silenced in the prodrug form (DR510-Pro) and selectively re-activated upon tumor-restricted enzymatic cleavage.
- ☐ **Ultra-low-dose efficacy:** In diverse mouse xenograft models, pronounced tumor regression was observed at extremely low doses (0.066-0.2 mg/kg), outperforming the Comparator.
- ☐ **Tumor-selective activation:** The intratumoral cleavage rate of DR510-Pro is ~20-fold higher than that of the Comparator, directly correlating with its superior therapeutic outcome.
- ☐ **Favorable systemic stability & safety:** DR510-Pro shows minimal cleavage in cynomolgus monkey peripheral blood; during a 4-week DRF study, only a transient, mild IL-6 increase was observed, with no clinical-pathology or histological abnormalities, supporting a wide safety margin for clinical development.
- ☐ The strategic dual-masking design of DR510 achieves a critical balance between therapeutic potency and safety. Supported by these robust preclinical data, DR510 is now advancing toward clinical translation for the treatment of EGFR-positive solid tumors.

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