DART® Molecules with Enhanced DR5 Agonistic Activity for Improved Cancer Cell Cytotoxicity

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Abstract Number: 2464

Abstract

Introduction: Death Receptor 5 (DR5) belongs to the tumor necrosis factor receptor superfamily. The target and its associated signaling pathway are preferentially active on cancer cells. DR5 elicits pro-apoptotic signal through receptor oligomerization upon binding of TRAIL or an agonistic mAb. Chemotherapy and radiation also can synergize with the DR5 pathway. Various therapeutics targeting DR5 have been generated but clinical outcomes have been generally disappointing. Here we describe optimization of the agonistic activity of anti-DR5 mAbs by enabling multivalent target engagement via engineering into Dual-Affinity Re-Targeting (DART®) molecules — covalently-linked Fv-based diabodies optimized for manufacturability and stability (1) whose modular nature facilitates tailoring avidity and PK properties to the specific needs of the application.

Methods: Anti-DR5 mAbs were selected from a panel of mAbs generated from cancer cell immunizations and characterized for binding properties. DARTs with varying anti-DR5 specificities, valency and incorporation of either a wild type IgG1 Fc or an Fc mutated to eliminate FcγR binding, were expressed in CHO cells and purified to homogeneity. DR5 DARTs and mAbs were characterized across a panel of cell lines including those derived from colorectal, lung, pancreatic, breast and prostate cancer and by IHC on normal and tumor tissue specimens.

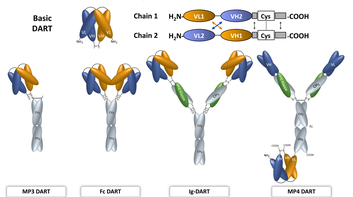
Results: mAbs from whole cancer cell immunizations displaying differential expression on both normal and cancer tissues were subjected to antigen identification and identified a subset with reactivity to DR5. Binding analyses revealed DR5 mAbs recognizing non-overlapping epitopes that do not block the TRAIL-DR5 interaction. Upon secondary cross-linking or when combined, the DR5 mAbs induced apoptosis across multiple cancer cell lines. Further potency enhancement independent of secondary cross-linking was obtained by the engineered multivalent DR5-targeting DARTs. Dose-dependent growth inhibition assay demonstrated that the DR5-targeting DARTs delivered significantly greater potency than TRAIL (≥100-fold) across a broad range of cancer cell lines. Furthermore, the DR5-targeting DARTs maintained the exquisite tumor/normal differential reactivity displayed by the parental DR5 mAbs from which they were derived. Combination of an HDAC inhibitor with DR5-targeting DARTs revealed maintenance of synergistic activity and potential to overcome pathway resistance.

Conclusion: Whole cancer cell immunizations yielded DR5 mAb candidates with desirable binding and functional properties. Through incorporation of the anti-DR5 mAb specificities into multivalent DART molecules, we have generated a new class of therapeutics that may overcome the limitations of existing DR5-based therapeutics. The data support the use of DR5-targeting DARTs to target this apoptotic pathway in multiple cancer cells.

Reference: (1) Johnson, et al. 2010. J Mol Bio. 399:436-49

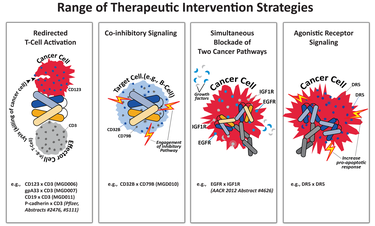
Dual-Affinity Re-Targeting (DART) Platform

Customizable Valency and Incorporation of Fc Domain



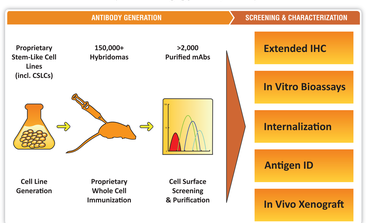
- Highly flexible platform for generating multi-specific molecules with antibody-like structure
- Engineered to accommodate any variable region sequence
- Enhanced manufacturability (multi-gram per liter in CHO cells) and long-term structural stability
- Ability to tailor half-life and incorporate desired valency: monovalent bispecific (MP3); bivalent bispecific or tetravalent (Fc-, Ig- or MP4 DART)

Range of Therapeutic Intervention Strategies



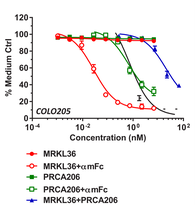
MacroGenics mAb Discovery Platform

>75 Cell Surface Cancer Targets Identified (including gpA33, DR5)



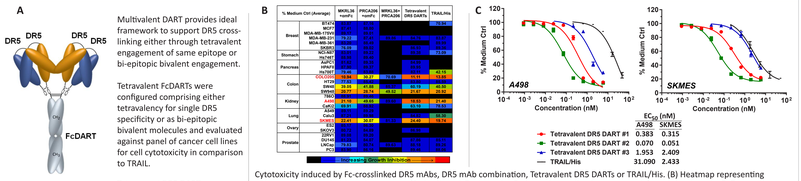
DR5 mAbs Selected from MacroGenics mAb library

- DR5 mAbs (MRKL36 and PRCA206) obtained from the MacroGenics mAb library
- Favorable IHC profile with Normal vs. Tumor tissues differential. Negative reactivity with multiple liver specimens
- Recognize non-overlapping epitopes on DR5 that are independent of TRAIL binding
- Range of binding affinities
- Cross-reactive with non-human primates counterpart
- DR5 mAbs are functionally inactive by themselves; however, potent cytotoxicity is induced upon secondary crosslinking or mAb combination in DR5-expressing cells



Results

Tetravalent DR5 DART Mediates Potent Cytotoxicity Independent of Cross-Linking



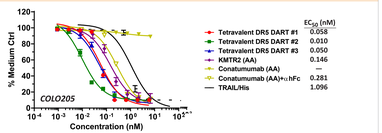
Multivalent DART provides ideal framework to support DR5 cross-linking either through tetravalent engagement of same epitope or bi-epitopic bivalent engagement.

Tetravalent FcDARTs were configured comprising either tetravalency for single DR5 specificity or as bi-epitopic bivalent molecules and evaluated against panel of cancer cell lines for cell cytotoxicity in comparison to TRAIL.

Tetravalent DR5 DARTs:
#1: MRKL36 x PRCA206
#2: MRKL36 x MRKL36
#3: PRCA206 x PRCA206

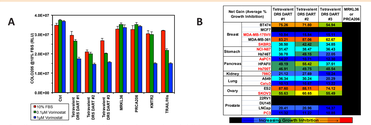
Cytotoxicity induced by Fc-crosslinked DR5 mAbs, DR5 mAb combination, Tetravalent DR5 DARTs or TRAIL/His. (B) Heatmap representing difference between treatment group and No-treatment Control after 2 days of incubation in presence of 1μg/ml treatment. (C) Dose response curve for cytotoxicity of A498 and SKMES cells. Tetravalent DR5 DARTs fully recapitulate cytotoxicity mediated by mAb Fc-crosslinking or mAb combination as reflected in maximum cytotoxicity across panel of Human cancer cell lines. Upto 10-100x enhanced cytotoxicity was observed when compared to TRAIL/His.

More Potent than Benchmark DR5 mAbs



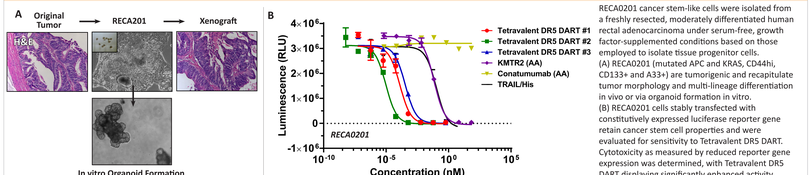
Dose response curve for cytotoxicity of COLO205 cells with Tetravalent DR5 DARTs, KMTR2 (AA), Conatumumab (AA) or TRAIL/His. KMTR (AA) and Conatumumab (AA) were constructed with Human IgG1 Fc doman with Ala/Ala mutation. Tetravalent DR5 DARTs display better potency than KMTR2 (AA) or Conatumumab (AA), with potency dependent on DR5 valency and specificity.

Sensitization with HDAC inhibitor



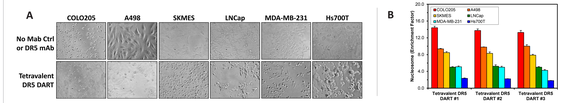
Studies have shown that Histone deacetylase inhibitor, such as Vorinostat, could overcome resistance to DR5 pathway. Consistent with previous findings, Vorinostat not only dose-dependently enhances Tetravalent DR5 DART cytotoxicity in COLO205 (A) but it also renders apoptosis-resistant cells responsive to Tetravalent DR5 DART treatment (B). Vorinostat does not modify the intrinsic activity of Parental DR5 mAb.

DR5 DARTs Mediate Cytotoxicity Against Colorectal Cancer Stem-like Cells



RECA0201 cancer stem-like cells were isolated from a freshly resected, moderately differentiated human rectal adenocarcinoma under serum-free, growth factor-supplemented conditions based on those employed to isolate tissue progenitor cells.
(A) RECA0201 (mutated APC and KRAS, CD44hi, CD133+ and A33+) are tumorigenic and recapitulate tumor morphology and multi-lineage differentiation in vivo or via organoid formation in vitro.
(B) RECA0201 cells stably transfected with constitutively expressed luciferase reporter gene retain cancer stem cell properties and were evaluated for sensitivity to Tetravalent DR5 DART. Cytotoxicity as measured by reduced reporter gene expression was determined, with Tetravalent DR5 DART displaying significantly enhanced activity compared to TRAIL and KMTR2.

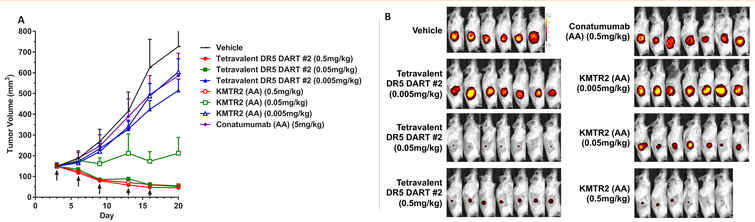
DR5 DARTs Induce Apoptosis



Evaluation across a panel of cancer cell lines revealed Tetravalent DR5 DARTs (1 μg/ml) were pro-apoptotic as exemplified by characteristic apoptotic cell morphology (A) and increased production of nucleosomes (B), cleaved PARP and active caspase 3 (data no shown) following a 4 hour incubation.

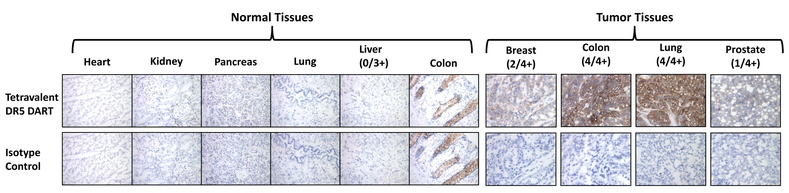
Results

Growth Inhibition of Tumor Xenografts in Mice



Inhibition of tumor growth by Tetravalent DR5 DART #2 in mice implanted with COLO205 cells. (A) Female hCD16A FOX N1 mice (n=7/group) were implanted SC with COLO205 cells on Day 0. On Day 3, mice were randomized based on tumor size and treated twice a week with indicated dose of Tetravalent DR5 DART #2, KMTR2 (AA), Conatumumab (AA) or Vehicle. Tumor volume was measured by caliper. (B) Whole body images were acquired on Day 20 with the IVIS Spectrum imaging system.

DR5 DARTs Display Favorable Normal/Tumor Differential by Immunohistochemistry



Immunohistochemical staining with Tetravalent DR5 DART (0.625 μg/ml) on fresh frozen normal and tumor tissues reveals an exquisite tumor to normal differential expression pattern. No reactivity was observed across the normal tissues examined (primary colon reactivity was deemed non-specific compared to isotype control). Four specimens per tumor indication (breast, colon, lung, prostate) were evaluated with the number of DR5 DART positive specimens indicated in parenthesis. Representative images of positive tumor specimens are presented.

Conclusions

- **Whole-cell cancer immunizations yielded DR5 mAbs with desirable binding and functional properties.**
- **Incorporation of anti-DR5 mAb specificities into multivalent DART molecules have resulted in a new class of therapeutics that may overcome limitations of existing DR5-based approaches.**
- **Tetravalent DR5 DART displays potent activity in vitro and in vivo, including efficacy against models of cancer stem-cells.**
- **Data support use of DR5-targeting DARTs to target this apoptotic pathway in multiple cancer types.**

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