Design and engineering of TRAIL fusion proteins for cancer therapy

Abstract # 3842

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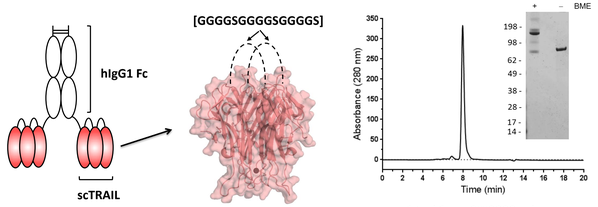
Abstract

Protein-based agonists of TRAIL receptors have shown remarkable preclinical efficacy but limited clinical response. The short circulating half-life of recombinant human TRAIL and the necessity of Fc-mediated clustering for potentiating agonistic DR4 and DR5 antibodies have been proposed to be major impediments to the clinical success of this class.

To address these limitations we have created Fc-scTRAIL, a single fusion polypeptide consisting of an IgG1 Fc region followed by three successive TRAIL monomers connected by two fifteen-amino acid linkers. Fc-scTRAIL showed increased apoptotic activity compared to TRAIL and agonist antibodies across multiple cell lines. Moreover, anti-Fc cross-linking did not improve Fc-scTRAIL mediated cell-killing suggesting that this hexavalent format maximizes death receptor activation.

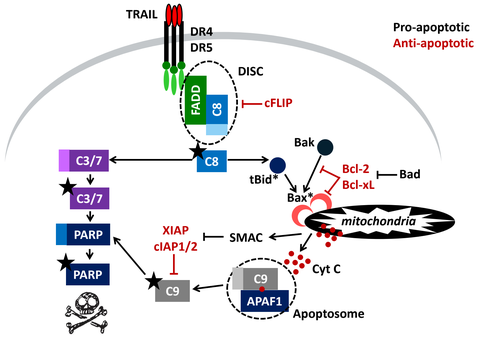
Wild-type Fc-scTRAIL exhibited a low T_M (52 °C) and 40-fold loss of activity in serum, an indication of protein instability. Subsequently, we performed directed evolution of TRAIL using yeast surface display and identified several mutations that stabilized the TRAIL trimer. Upon transfer of these mutations into the Fc-scTRAIL format, we observed a dramatic increase in T_M (68 - 72 °C) and significantly reduced activity loss (< 4-fold) in serum. This translated to an extended half-life (39 hours) in mice for T191, our most stabilized Fc-scTRAIL variant. Finally, in COLO205 xenograft model, T191 showed much greater efficacy than TRAIL at an equivalent dose.

Fc-scTRAIL: A single polypeptide chain TRAIL fused to the Fc of human IgG1



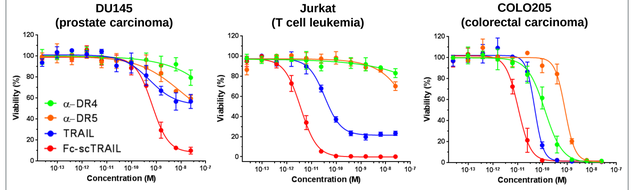
- TSKgel SuperSW3000 column

TRAIL apoptotic pathway

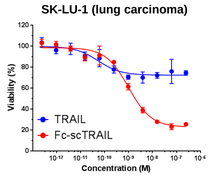


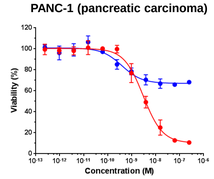
Fc-scTRAIL is a potent inducer of apoptosis

Fc-scTRAIL is more active than rhTRAIL or agonistic antibodies

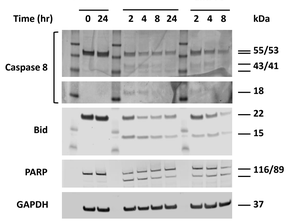


Fc-scTRAIL induces apoptosis in TRAIL-resistant cell lines





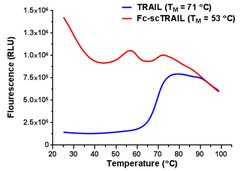
Fc-scTRAIL induced caspase-8 activation is independent of Fc-mediated cross-linking



- For the immunoblot analysis, DU145 cells were treated with 10 nM of Fc-scTRAIL in presence or absence of anti-Fc (12.5 nM) and lysed at times indicated
- Viability was measured using CellTiter-Glo (Promega) after 24 hrs of treatment

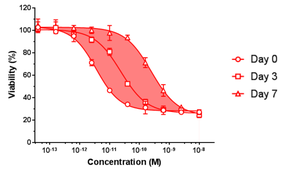
Liabilities of Fc-scTRAIL design

Low T_M compared to native TRAIL



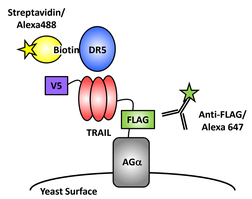
- Thermal shift was measured by differential scanning fluorimetry

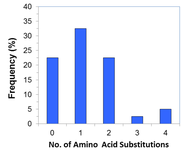
40-fold loss in activity upon extended incubation with serum



- Fc-scTRAIL was incubated in 90% mouse serum at 37 °C and then used to treat HCT116 cells.

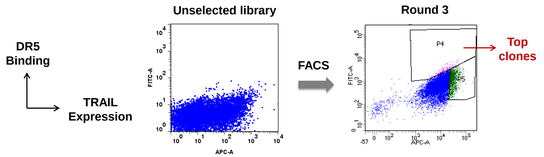
Addressing liabilities of Fc-scTRAIL through directed evolution of TRAIL using yeast display





- Random mutagenesis library created using error-prone PCR
- Library size 1.1e8

Variants with improved DR5 binding observed after 3 rounds of FACS

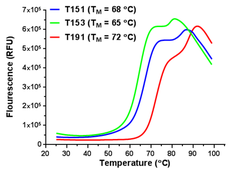


Fc-scTRAIL variants have improved T_M and retain activity in serum

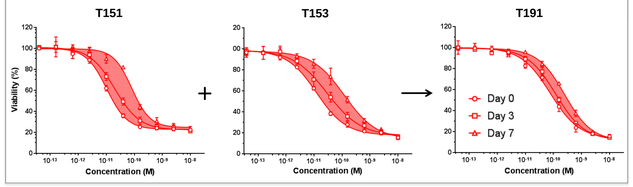
- Top yeast clones for DR5 binding were expressed in HEK293 cells in Fc-scTRAIL format
- Mutations from top variants, T151 and T153, were combined to create T191

	Amino acid substitutions
T151	N228S/I247V
T153	R130G/I247V
T191	R130G/N228S/I247V

Variants have significantly increased T_M

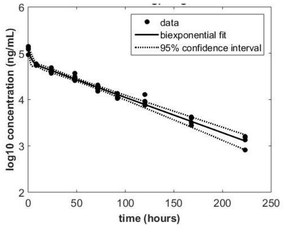


Additive effect of mutations result in < 4-fold activity loss in serum



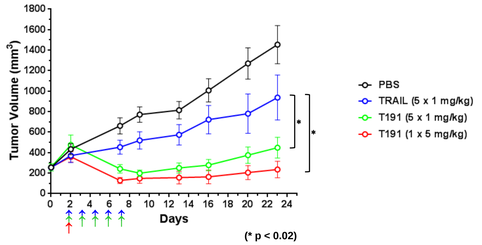
In vivo properties of Fc scTRAIL variant T191

T191 has enhanced PK compared to previous TRAIL-based molecules



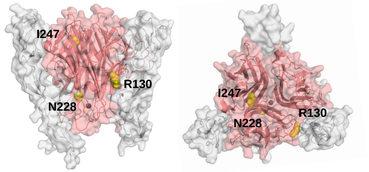
	Terminal half-life	Reference
TRAIL	3.6 min	Kelley et al. 2001
scTRAIL	35 min	Schneider et al. 2010
scTRAIL-Fc	28 hrs	Gieffers et al. 2013
T191	**39 hrs**	

T191 shows superior efficacy compared to TRAIL in a COLO205 model



(* p < 0.02)

Position of mutations in TRAIL



- Mutations (yellow spheres) are indicated in the TRAIL (red) and DR5 (grey) complex
- N228 and I247 are involved in TRAIL protomer-protomer interactions
- R130 is in the TRAIL/DR5 interface

Conclusions

- **Fc-scTRAIL is more potent than previous clinical agonists of apoptosis**
- **Stabilizing mutations have increased the stability of Fc-scTRAIL resulting in improved half-life and activity *in vivo***
- **Stabilized Fc-scTRAIL has the potential to improve clinical outcomes**