Poster Number: 4460, AACR Annual Meeting 2020, June 22 - 24, Virtual Meeting II, Poster Session Title: Combination Immunotherapies 3

Novel bispecific molecules combining HERA-CD40L with anti-CEA or with anti-PD-L1 for targeting

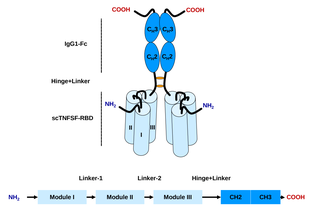
Meinolf Thiemann, Katharina Billian-Frey, Matthias Schröder, Jaromir Sykora, David M. Richards, Christian Merz, Mauricio Redondo Müller, Julian P. Sefrin, Karl Heinonen, Christian Gieffers, and Oliver Hill

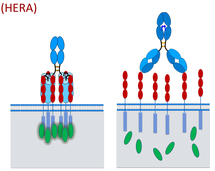
Research & Development, Apogenix AG, Im Neuenheimer Feld 584, 69120 Heidelberg, Germany

Contact: meinolf.thiemann@apogenix.com

HERA technology platform

Apogenix Hexavalent TNF superfamily Receptor Agonists (HERA)





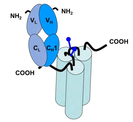
HERA compounds are superior to antibodies

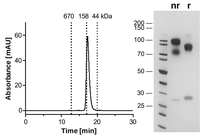
	HERA Platform	Agonistic Antibodies
Mimics natural ligand	YES	NO
Fcγ-receptor independent signaling	YES	NO
Half-life	6 – 84 hours	1 – 4 weeks
Survival of receptor expressing cells	YES	(NO)

- Optimal spatial organization of ligand induced receptor clustering mimicking the natural binding mode
- Agonistic activity independent of additional cross-linking by Fcγ receptors
- Fc is silenced to ensure true agonistic activity rather than mixed mode action

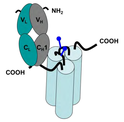
Novel bispecifics derived from the HERA technology platform

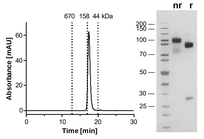
aPD-L1-scCD40L(tri)



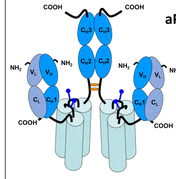


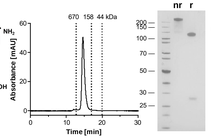
aCEA-scCD40L(tri)



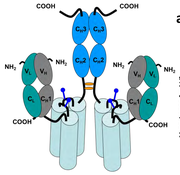


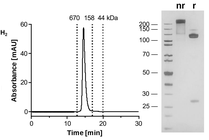
aPD-L1-HERA-CD40L(hex)



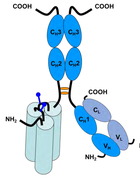


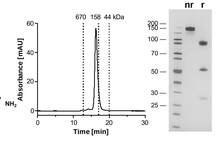
aCEA-HERA-CD40L(hex)





aPD-L1-scCD40L(tri)-SAB





SAB: single arm bispecific

Bispecifics production

Features: Trivalent (tri) or hexavalent (hex) TNF-SF receptor agonist combined with Fab targeting PD-L1, CEA (or other targets), silenced Fc or no Fc

USP: CHO-S based fed batch suspension culture

DSP: Affinity chromatography followed by SEC-based polishing or two step affinity chromatography (lab-scale process)

Analytical HPLC-SEC:

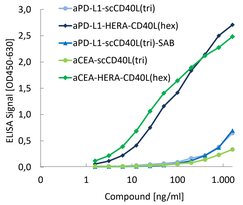
The bispecific molecules display a single peak with no aggregates or a low degree of aggregates respectively

SDS-PAGE:

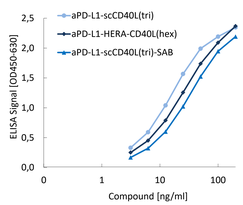
Non-reducing (nr) and reducing (r) SDS-PAGE confirm the molecular weight and the high level of purity of the bispecific molecules

Novel bispecifics bind to their respective targets

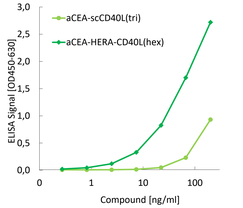
Binding to human CD40 (ELISA)



Binding to human PD-L1 (ELISA)



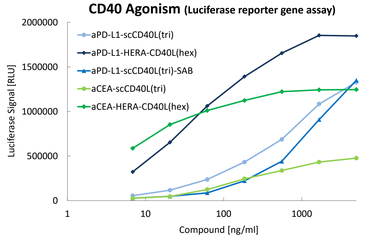
Binding to human CEA (ELISA)



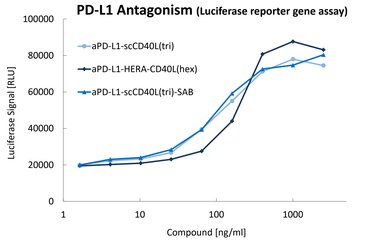
- Binding to CD40 is consistent with the layout of the bispecific molecules: hexavalent molecules [aPD-L1-HERA-CD40L(hex), aCEA-HERA-CD40L(hex)] display stronger binding than the trivalent molecules [aPD-L1-scCD40L(tri), aCEA-scCD40L(tri), aPD-L1-scCD40L(tri)-SAB]
- Binding to PD-L1 is comparable for the three bispecific molecules investigated
- Binding of aCEA-HERA-CD40L(hex) to CEA is stronger than that of aCEA-scCD40L(tri)

Novel bispecifics show biological activity on cells

CD40 Agonism (Luciferase reporter gene assay)



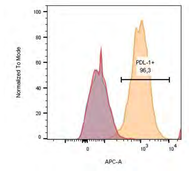
PD-L1 Antagonism (Luciferase reporter gene assay)



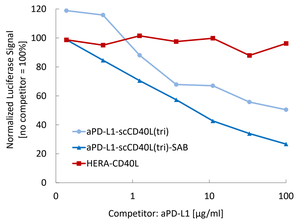
- PD-L1 targeting of bispecific molecules clearly contributes to CD40 agonism in a cellular assay: bispecifics comprising an aPD-L1 Fab domain are clearly more active than comparable molecules which target CEA instead
- PD-L1 antagonism is comparable for the three bispecific molecules investigated

PD-L1 targeting of bispecifics clearly contributes to CD40 agonism

CD40 cells from the Luciferase Reporter gene assay are uniformly positive for PD-L1 (Flow cytometry)



CD40 Agonism (Luciferase reporter gene assay): Competition with an anti-PD-L1 antibody

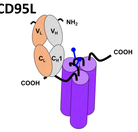


The CD40 Luciferase reporter gene assay was carried out employing constant concentrations of aPD-L1-scCD40L(tri) [200 ng/ml], aPD-L1-scCD40L(tri)-SAB [200 ng/ml] and HERA-CD40L [25 ng/ml] together with increasing concentrations of an anti-PD-L1 mAb [up to 100 µg/ml]. Luciferase signals were normalized to CD40 agonistic activity in the absence of competing anti-PD-L1 mAb [=100%]. HERA-CD40L serves as a control since it does not target PD-L1 (its activity remained unchanged in the presence of anti-PD-L1 mAb).

- The contribution of PD-L1 targeting to CD40 agonism was confirmed: cellular activity of bispecifics comprising an aPD-L1 Fab domain is clearly reduced by competition with anti-PD-L1 mAb

Further bispecifics derived from the HERA technology platform

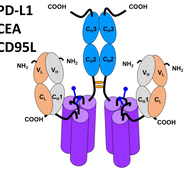
aPD-L1
aCEA
aCD95L



scCD40L(tri)
scCD27L(tri)
scGITRL(tri)
sc4-1BBL(tri)
scLIGHT(tri)

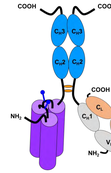
Realized combinations
aPD-L1-scCD40L(tri)
aCEA-scCD40L(tri)
aCD95L-scCD40L(tri)

aPD-L1
aCEA
aCD95L



HERA-CD40L(hex)
HERA-CD27L(hex)
HERA-GITRL(hex)
HERA-4-1BBL(hex)
HERA-LIGHT(hex)

Realized combinations
aPD-L1-HERA-CD40L(hex)
aCEA-HERA-CD40L(hex)
aCD95L-HERA-CD40L(hex)
aCEA-HERA-CD27L(hex)
aCEA-HERA-GITRL(hex)



scCD40L(tri)
scCD27L(tri)
scGITRL(tri)
sc4-1BBL(tri)
scLIGHT(tri)

aPD-L1
aCEA
aCD95L

Realized combinations
aPD-L1-scCD40L(tri)-SAB, aCD95L-scCD40L(tri)-SAB
aPD-L1-scCD27L(tri)-SAB, aCD95L-scCD27L(tri)-SAB
aPD-L1-scGITRL(tri)-SAB, aCD95L-scGITRL(tri)-SAB
aPD-L1-sc4-1BBL(tri)-SAB, aCD95L-sc4-1BBL(tri)-SAB
aPD-L1-scLIGHT(tri)-SAB, aCD95L-scLIGHT(tri)-SAB

- Consistent producibility of the bispecific molecules was demonstrated (see realized combinations) and high purity of the proteins was confirmed as well as low content of aggregates
- Most of the realized combinations were profiled with respect to target binding and cellular in vitro activity demonstrating the functionality of the molecules

Summary & Outlook

Bispecific molecules derived from the HERA technology platform:

- can be produced as homogenous, aggregate-free / aggregate-low protein batches with good cellular in vitro activity
- were shown to exert improved agonistic activity due to the targeting domain
- are amenable for inclusion of any desired Fab-fragment for targeting in combination with any desired HERA-ligand or scTNF-SF-ligand

Ongoing and future studies explore the efficacy of these bispecifics in mouse-tumor models

Abstract

Introduction: CD40 ligand is a member of the TNF superfamily and a key regulator of the immune system. Its cognate receptor CD40 is expressed on antigen-presenting cells and on many tumor types, and has emerged as an attractive target for immunological cancer treatment. We have shown previously, that hexavalent HERA-CD40L is a potent CD40 agonist which is clearly superior over anti-CD40 benchmark antibodies and able to establish single agent anti-tumor immune responses both *in vitro* and *in vivo*. Since this compound qualifies as an ideal candidate for combinatorial cancer treatments we have created bispecific molecules by adding antibody derived targeting domains to the HERA-CD40L scaffold. These bispecific fusion proteins combine the potent co-stimulatory CD40-agonist with additional functionalities to enable tumor targeting and/or additional immunomodulatory activities. To evaluate the different fusion protein formats in principle, the tumor associated antigens CEA and PD-L1 were chosen as targets. In addition to the hexavalent targeted HERA-CD40L, trivalent targeted fusion proteins employing the single-chain CD40L (scCD40L) as building block were created.

Materials, Methods & Results: Anti-CEA-HERA-CD40L, anti-CEA-trivalent scCD40L, anti-PD-L1-HERA-CD40L and anti-PD-L1-trivalent scCD40L were produced in CHO-S cells and purified resulting in highly pure non-aggregating protein lots as demonstrated by SDS-PAGE and HPLC-SEC. ELISA assays confirmed the specific binding to their targets – CD40 and CEA or CD40 and PD-L1, respectively. Employing a CD40 Luciferase reporter gene assay, hexavalent anti-CEA-HERA-CD40L showed a strong agonistic activity which was clearly superior to the anti-CEA-trivalent scCD40L- construct. Similarly, hexavalent anti-PD-L1-HERA-CD40L showed a strong agonistic activity in this assay which also was clearly superior to the anti-PD-L1-trivalent scCD40L construct. A PD-1/PD-L1 Luciferase reporter gene assay assessing the cellular activity of compounds interfering with PD-1/PD-L1 binding showed a clear activity for anti-PD-L1-HERA-CD40L. As expected for an assay assessing antagonistic activities, the activity of hexavalent anti-PD-L1-HERA-CD40L was in the same range as a reference anti-PD-L1 antibody and the anti-PD-L1-trimeric scCD40L- construct.

Conclusion: Based on the in vitro data presented, the bispecific molecules combining HERA-CD40L with tumor targeting (anti-CEA) or with a checkpoint-blockade inhibitor (anti-PD-L1) are promising therapeutic approaches to promote anti-tumor immune responses.