Trastuzumab and C6.5 diabody armed with deBouganin overcome drug resistance to ADCs comprised of anti-microtubule agents

S. Chooniedass[a], R. L. Dillon[a], A. Premsukh[a], G. P. Adams[b], G. C. MacDonald[b], J. Cizeau[a]

[a]Viventia Bio Inc. Winnipeg, MB, Canada; [b]Eleven Biotherapeutics Cambridge, MA, USA

Abstract # 79

ABSTRACT

DeBouganin (deB) is a de-immunized form of bouganin, a Ribosome Inactivating Protein (RIP) that when internalized blocks protein synthesis thereby leading to cell death. When conjugated to trastuzumab (T-deB) or genetically attached to the C6.5 diabody, deBouganin was more potent than DM1 and unaffected by mechanisms of resistance to which DM1 is susceptible. To further highlight the differentiating mechanism of action of deBouganin, HCC1419 and BT-474 tumor cells that survived T-DM1 or trastuzumab-MMAE (T-MMAE) treatment in vitro were re-exposed to T-DM1, T-MMAE, or treated with T-deB or an anti-HER2 C6.5 diabody-deBouganin fusion protein. C6.5 diabody-deBouganin and T-deB were potent against HCC1419 and BT-474 cells surviving T-DM1 or T-MMAE treatment. However, the surviving cell populations were resistant to T-DM1, T-MMAE, DM1, MMAE and taxol treatment. In addition, cross-resistance was seen against trastuzumab-duocarmycin which contains a payload with a cell cycle independent mechanism of action. The contribution of multi-drug resistance, Bcl-2 family members and other survival pathways accounting for the resistant phenotype will be discussed. Overall, the data suggest that treatment with chemotherapeutics or ADCs comprised of small molecule compounds such as anti-microtubule agents, can lead to the outgrowth of tumor cells resistant to similar agents. In contrast, antibodies and antibody fragments armed with deBouganin can overcome these mechanisms of resistance and may therefore represent a more effective treatment option.

BACKGROUND

Bouganin is a plant type 1 RIP isolated from *Bougainvillea spectabilis* Willd that demonstrates potent antitumor activity when delivered in the context of an antibody or antibody fragment [1]. A de-immunized variant of bouganin, deBouganin, has been created through the removal of T-cell epitopes, thus allowing for repeat systemic administration and thereby addressing one of the major challenges facing immunotoxins. A study comparing the biological activity of deBouganin conjugated to trastuzumab (T-deB) and T-DM1 highlighted deBouganin's mechanism of action versus the small molecule payload. Not only was a greater potency for deBouganin observed as compared to DM1 for the majority of high HER2 expressing cell lines, T-deB cytotoxicity was unaffected by a number of drug resistance mechanisms to which T-DM1 was susceptible, including MDR efflux pumps and modulation of apoptotic processes [2]. Similar to T-deB, deBouganin genetically linked to an anti-HER2 C6.5 diabody (deB-C6.5-diab) was more potent than T-DM1 and either more or equally as potent as T-MMAE against most HER2 3+ tumor cell lines [3]. To further highlight the differentiating mechanism of action of deBouganin, the potency of T-deB and deB-C6.5-diab was assessed against HCC1419 and BT-474 tumor cells surviving T-DM1 or T-MMAE treatment. The mechanisms of resistance enabling cells to survive T-DM1 or T-MMAE treatments were further studied.

METHODS

Growth profile. To measure the viability of tumor cells surviving a single 5-day exposure to drug, tumor cells were treated with 10 nM deB-C6.5-diab, T-DM1 or T-MMAE. Cells surviving the treatment were trypsinized and reseeded at 5,000 cells per well in a 96 well plate. Viability was measured using an MTS assay and reported as $O.D._{490}$ absorbance values.

Potency. Tumor cells surviving a single 5-day exposure to 10 nM T-DM1 or T-MMAE were reseeded and treated for another 5 days with either deB-C6.5-diab, T-deB, T-DM1, T-MMAE, DM1, MMAE, trastuzumab-duocarmycin (T-duo), duocarmycin (duo), taxol or anti-EpCAM scFv genetically linked to a truncated form of *Pseudomonas* exotoxin A (ETA) (scFv-ETA) over a range of concentrations. Viability was reported relative to that of the non-treated control cells.

MDR assessment. Cell viability was assessed using concomitant treatments with PSC833 (MDR1 inhibitor), MK571 (MRP1 inhibitor), and Ko143 (BCRP inhibitor). The inhibitors were held at a fixed concentration in combination with 10 nM T-DM1 or T-MMAE. Doxorubicin (MDR1 and MRP1 substrate) and irinotecan (BCRP substrate) were used as controls.

Western blot analysis. Control or 5-day treated cell extracts were prepared in lysis buffer and 60 µg was loaded on a SDS-PAGE gel under reducing conditions. Antibodies for immunoblots included anti-phospho-AKT (Ser473), anti-phospho-JNK/SAPK (Thr183/Tyr185), anti-phospho-p38 MAPK (Thr180/Tyr182), anti-MDR1, anti-MRP1, anti-BCRP, anti-Mcl-1, anti-Bcl-xL and anti-β-actin. All membranes were incubated with horseradish peroxidase-conjugated secondary antibodies and visualized using enhanced chemiluminescence.

RESULTS

Growth Profile of Treated Cells

- **No significant proliferation is measured with any of the deB-C6.5-diab treated tumor cell lines**
- **HCC1419 cells surviving T-DM1 or T-MMAE treatment continue to grow while BT-474 surviving cells remain viable and show minimal growth**

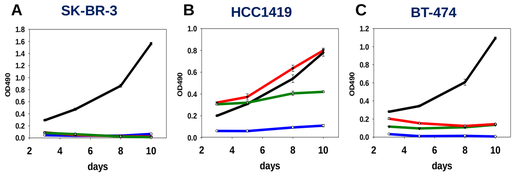

Figure 1: Growth profiles of SK-BR-3 (A), HCC1419 (B), or BT-474 (C) cells surviving deB-C6.5-diab (blue), T-DM1 (red), T-MMAE (green) treatment or untreated cells (black).

DeBouganin is Potent against T-DM1 and T-MMAE Treated Cells

- **T-DM1 or T-MMAE-treated BT-474 and HCC1419 cells are resistant to further T-DM1 or T-MMAE exposure (Figure 2A and 2B)**
- **T-deB and deB-C6.5-diab are cytotoxic against T-DM1 or T-MMAE treated cells (Figure 2C and 2D)**

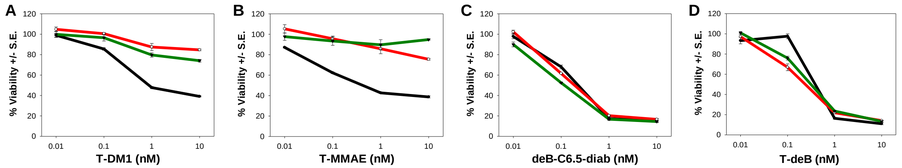

Figure 2: Cytotoxicity of T-DM1 (A), T-MMAE (B), T-deB (C), and deB-C6.5-diab (D) against HCC1419 cells (black), HCC1419 cells surviving T-DM1 (red) and HCC1419 cells surviving T-MMAE (green) treatment.

T-DM1 and T-MMAE Treated Cells Demonstrate Cross Resistance

- **T-DM1 or T-MMAE-treated BT-474 and HCC1419 cells exhibit cross resistance to T-duocarmycin and taxol**

Table 1: Potency (IC_{50}) against untreated (NT), T-DM1 or T-MMAE treated BT-474 and HCC1419 cells

	BT-474			HCC1419		
	NT	T-DM1 treated	T-MMAE treated	NT	T-DM1 treated	T-MMAE treated
deB-C6.5-diab	0.07 (0.02)	0.33 (0.27)	0.11 (0.01)	0.15 (0.02)	0.19 (0.01)	0.17 (0.05)
T-deB	0.18 (0.005)	0.9	0.425 (0.175)	0.25	0.193 (0.038)	0.27 (0.03)
T-DM1	0.85 (0.25)	>10	>10	1.9 (0.9)	>10	>10
T-MMAE	0.04 (0.01)	>10	>10	5.7 (5.1)	>10	>10
T-Duo	0.4 (0.1)	>10	9.95 (5.05)	0.28 (0.03)	4.65 (1.85)	>10
scFv-ETA (pM)	0.09 (0.03)	0.18 (0.0)	0.36 (0.04)	0.2 (0.0)	0.3 (0.0)	0.28 (0.03)
DM1	17 (8)	>100	>100	135 (65)	>100	>100
MMAE	0.57 (0.17)	>100	>100	7 (3)	>100	>100
Taxol	18 (3)	>1000	>1000	>1000	>1000	>1000
Duocarmycin	0.31 (0.08)	5.45 (1.45)	3.05 (0.45)	0.65 (0.05)	3.55 (0.45)	4.95 (0.05)

IC_{50} values expressed in nM (unless indicated) are the mean of a minimum of 2 representative experiments with 3 replicates per dilution. Values in parentheses indicate the S.E.

Mechanisms of resistance in T-DM1 and T-MMAE Treated Cells

- **Potency of both doxorubicin and irinotecan is completely or partially restored in the presence of MDR1, MRP1 or BCRP inhibitors**
- **Co-treatment with inhibitors is largely ineffective at restoring T-DM1 or T-MMAE cytotoxicity**

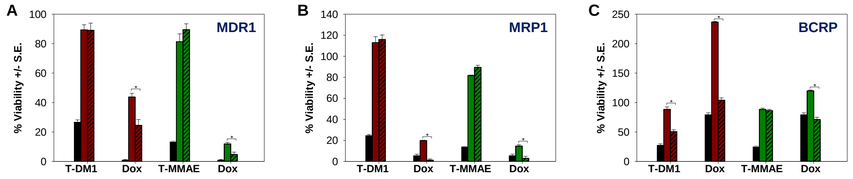

Figure 3: Role of MDR proteins. Cytotoxicity of T-DM1, T-MMAE, doxorubicin or irinotecan was measured against untreated (black), T-DM1 (red) or T-MMAE (green) surviving BT-474 cells with concomitant treatment with 1 µM MDR1 inhibitor PSC833 (A), 30 µM MRP1 inhibitor MK571 (B) or 10 µM BCRP inhibitor Ko143 (C) (hashed bars).

- **Higher MDR expression in treated tumor cells as compared to untreated cells**
- **Higher JNK phosphorylation in treated BT-474 cells**
- **Upregulation of Bcl-xL in treated BT-474 cells**
- **Lower levels of phospho-AKT in treated cells**
- **No change in caveolin and Mcl-1 expression in treated cells**

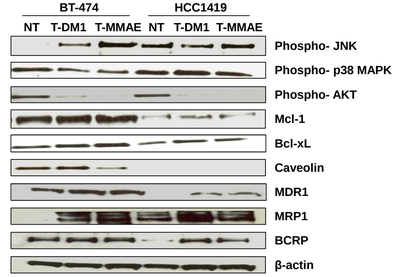

Figure 4: Differential protein expression and phosphorylation status between untreated (NT) and cells surviving T-DM1 or T-MMAE treatment. β-actin levels were monitored to ensure equal loading.

SUMMARY

- **Treatment with either T-DM1 or T-MMAE results in an outgrowth of cells with a similar pattern of drug resistance.**
- **T-DM1 or T-MMAE resistant tumor cells demonstrate cross resistance to T-duocarmycin and taxol.**
- **Multifaceted resistant phenotype includes over-expression of MDR proteins and a differential phosphorylation status of proteins involved in cell survival pathways.**
- **DeBouganin either conjugated to trastuzumab or genetically fused to C6.5 diabody overcomes these mechanisms of resistance.**

References

- Cizeau, J. *et al.* J Immunother. 2009, 32(6), 574-584.
- Dillon, R.L. *et al.* J Immunother. 2016, 39(3), 117-126.
- Chooniedass, S. *et al.* Molecules. 2016, 21(12).

Contact: gregoryadams@elevenbio.com

Poster 79, American Association for Cancer Research, Session: Experimental and Molecular Therapeutics, Antibody Technology, Sunday Apr 2, 2017 from 1:00 to 5:00 PM.

ELEVEN BIOTHERAPEUTICS