Melflufen Efficacy in Multiple Myeloma with *TP53* Aberrations

Poster
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BACKGROUND

Multiple myeloma (MM) is an incurable plasma cell malignancy characterized by clonal evolution and heterogeneous genetic abnormalities. Deletion of the short arm of chromosome 17 (del17p) harboring *TP53* is a high-risk abnormality associated with aggressive disease and therapy resistance.

OBJECTIVES

To test *in vitro* and *ex vivo* efficacy of a peptide-drug conjugate melphalan flufenamide (melflufen), a peptide-drug conjugate currently in phase 3 clinical trials for relapsed/refractory multiple myeloma (RRMM), in isogenic myeloma p53-abrogated cell line model and patient samples and elucidate molecular pathways involved in the response.

METHODS

Efficacy of melflufen versus melphalan was tested in the AMO-1 cell line, which displays biallelic wild-type *TP53* and retains aspects of a functional p53 system, and AMO-1 clones with either complete loss of p53 or expressing point-mutated p53 protein (R282W hotspot mutation). We assessed toxicity and apoptosis using Annexin V and alamarBlue assays.

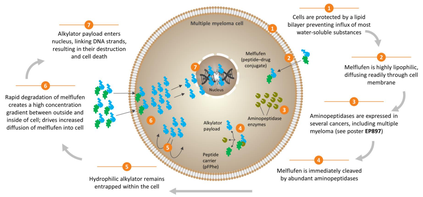
Ex vivo sensitivity to the drugs was examined by measuring CD138+CD38+ plasma cell viability in primary bone marrow samples from MM patients with confirmed del17p or *TP53* mutations. Cell viability was assessed using Annexin V & 7-AAD with high throughput flow cytometry

RNAseq analysis of AMO-1 wt and AMO1 *TP53* -/- cells was preformed 2h after melphalan and melflufen treatment to identify differential responses to the drugs. Extended time course analysis is currently ongoing.

We also assessed response rates* in a sub-population of patients with confirmed del17p from HORIZON (OP-106), a phase 2 study evaluating the efficacy of melflufen plus dexamethasone in patients with RRMM (NCT02963493). **Data cutoff July 2019*

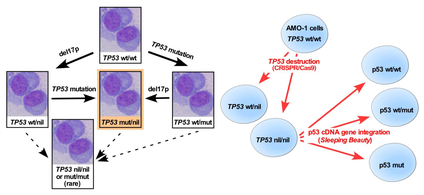
RESULTS

Figure 1. Melflufen mechanism of action



pFPhe (p-fluoro-phenylalanine); Ref 1-5

Figure 2. Left: Illustrative model of the acquisition of *TP53* lesions commonly seen in MM cells.

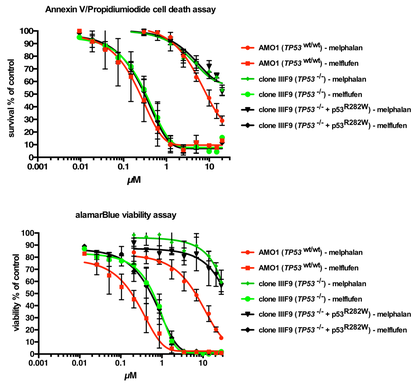


Generation of an isogenic cell line model with *TP53* abrogation. *TP53* locus in AMO-1 cells was targeted by CRISPR/Cas9 followed by the establishment of p53 cDNA gene expression in *TP53* nil/nil clones via Sleeping Beauty generating p53 wt/wt, p53 wt/mut and p53 nil/mut scenarios [6].

Table 1: Response rates* in a subpopulation of patients with confirmed del17p from HORIZON (NCT02963493) **Data cutoff July 2019*

NCT02963493 HORIZON			
Group	**N***	**ORR (%)**	**CI 95%**
del17p subgroup	15	20	4.3-48.1
Total population	136	26.2	18.8-34.6

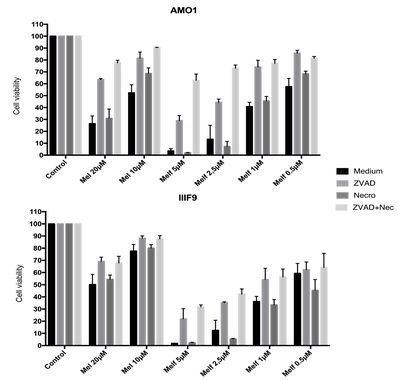
Figure 3. Melflufen vs. melphalan effects in the AMO-1 *TP53* model system assessed 72h after treatment with increasing doses of the drugs.



Whereas loss of p53 functionality strongly impaired melphalan induced cell death in the AMO-1 MM cell line model system, treatment with melflufen showed superior efficacy and was effective independent of the respective *TP53* status.

In the cell viability assay (alamarBlue) at the low melflufen dosages, wild-type cells showed higher sensitivity, but in contrast to melphalan this effect was overcome by a slight dose increase, due to the much steeper dose-effect relationships in the p53 deficient sublines.

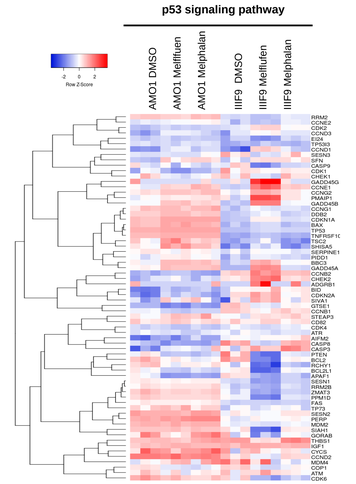
Figure 4. Effects of apoptosis and necroptosis inhibition on melflufen and melphalan efficacy in the AMO-1 *TP53* model system.



Cells were preincubated for 1h with 50 μM apoptosis inhibitor ZVAD and/or 100 μM necroptosis inhibitor necrostatin-1 before addition of the respective drugs. AnnexinV-PI staining was performed after 48h.

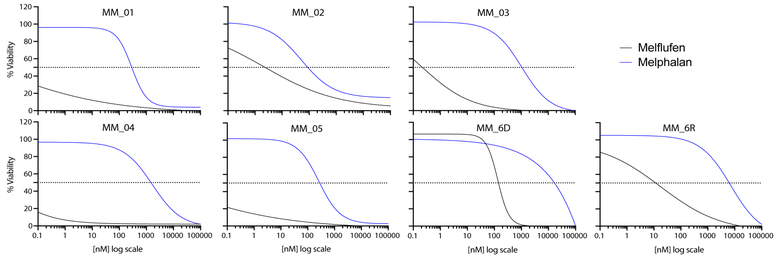
Inhibition of apoptosis and necrosis could to greater extent revers cytotoxic effect of melflufen in AMO-1 wt than in AMO-1 IIIF9 *TP53*-/- null cells, suggesting that other p53-independent cell death mechanisms are active in these cells.

Figure 5. RNAseq analysis of AMO-1 wt and AMO-1 IIIF9 *TP53*$^{-/-}$ clone 2 hours after treatment with IC90 dose melflufen (0.5 and 2.5 μM)



Analysis of early response shows that compared to melphalan, melflufen strongly induces *GADD45* genes as well as proapoptotic *BBCR* in AMO-1 IIIF9 *TP53*-/- clone but to the less extent in AMO-1 wt.

Figure 6. *Ex vivo* sensitivity to melflufen and melphalan of CD138+CD38+ plasma cells in primary bone marrow samples from MM patients assessed by flow cytometry. **Table:** Patient characteristics and *TP53* status

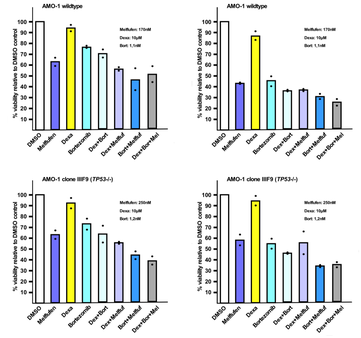


Patient ID	Disease stage	Sex	Age	Line of treatment	Cytogenetics	del17p (%) FISH	TP53 mutation	Melflufen EC50 [nM]	Melphalan EC50 [nM]
MM_01	RRMM	Male	78	4	t(11;14), del(13q), 1q gain		TP53	0.001414	290.1
MM_02	RRMM	Male	67	1	**del(17p13)**, 1q gain, Trisomy 11, 1p36 loss, del14q32	79	TP53	2.185	51.64
MM_03	RRMM	Male	80	5	**del(17p13)**, t(11;14), del(13q), 1q gain	35	NA	0.2335	1059
MM_04	RRMM	Male	63	2	**del(17p13)**, 1q gain, 1p36 loss, Trisomy 9, t(14q32), Trisomy 5, Trisomy 15	68	TP53	0.002919	1671
MM_05	RRMM	Female	63	1	**del(17p13)**, del(13q), 1q gain, t(14;20)	76	Not detected	0.0002194	259.2
MM_06D	NDMM	Male	77	0	**del(17p13)**, del(13q), t(4;14), del16q23	100	Not detected	133.5	> 10 000*
MM_06R	RRMM	Male	79	5	**del(17p13)**, del(13q), t(4;14), del14q32	90	NA	17.74	7207

* Ambiguous fit

Melflufen shows high and superior efficacy over melphalan in all samples tested, including "double hit" samples MM_02 and MM_4 carrying del(17)p and *TP53* mutations. Interestingly, increased sensitivity to melflufen was observed in the MM_06R RRMM sample compared to the NDMM sample form the same patient.

Figure 7. Treatment of AMO-1 wt and *TP53*-/- AMO-1 cells with melflufen, bortezomib and dexamethasone or the combination, and viability assessment by alamarBlue-assay after 72h.



Drug concentrations were chosen such that they fall within the 40-70% effect ranges for either melflufen or bortezomib alone for each cell line. Shown are two independent sets of experiments.

Additive effects of melflufen in combination with bortezomib was observed in both lines, independent of their *TP53* status.

CONCLUSION

- Melflufen can elicit myeloma cell death regardless of p53 status, and thus overcome p53-deficiency-mediated melphalan resistance.
- Besides apoptosis and necroptosis, melflufen can possibly trigger additional p53-independent cell death mechanisms in myeloma cells.
- Melflufen demonstrates high ex vivo efficacy in MM patient-derived plasma cells harboring del17p and *TP53* mutations.
- Melflufen provides an additive effect when combined with bortezomib regardless of *TP53* status.
- Melflufen might provide a valuable therapeutic option for del(17)p RRMM subpopulation of patients that is usually difficult to treat.

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DISCLOSURES

AS: Oncopeptides AB: Employment. **UM:**no conflicts of interest to report **JA:** Oncopeptides AB: Employment. **FL:** Oncopeptides AB: Employment. **JJM and MEH:** no conflicts of interest to report. **RCB:** no conflicts of interest to report **NNN:** Oncopeptides AB: Consultancy. **PR:** honoraria from Celgene and Janssen; consulting and advisory role with Celgene and Janssen; research funding from Celgene and Bristol-Myers Squibb. **PR**: consulting/advisory role with Oncopeptides and research funding from Oncopeptides. **MVM:** honoraria from Janssen, Celgene, Amgen, and Takeda; consulting/advisory role with Janssen, Celgene, Amgen, Takeda, GSK, AbbVie, and Oncopeptides. **CAH:** Celgene: Research funding; Kronos Bio: Research funding; Novartis: Research funding; Oncopeptides: Research funding; Orion Pharma: Research funding. **TS:** Oncopeptides: Research funding

Melflufen is an abbreviated form of the international non-proprietary name (INN) melphalan flufenamide, an investigational product not yet approved for commercial use in any market globally