Evaluation of the activity of IMiDs in MM proliferating cells

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ABSTRACT

Background: Treatment of multiple myeloma (MM) has evolved from nonspecific chemotherapeutic agents to novel targeted therapy. Among these new treatment approaches, the immunomodulatory drugs (IMiDs) represent a good therapeutic option for many MM patients. Several mechanism of action has been proposed to explain the direct and indirect effect of IMiDs, such as antiangiogenic, proapoptotic, antiproliferative and immunomodulatory. Although there are 3 IMID drugs approved (Thalidomide, Lenalidomide, Pomalidomide), in vitro assays to capture the IMiDs activities are still in development. Precision medicine tests evaluating these drugs could represent a valuable tool to assess the subgroup of patients who could benefit from these treatments.

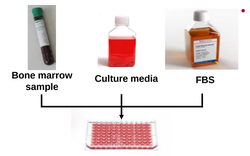
Aim: The goal of this study is to design an ex vivo model with the PharmaFlow technology, that allows to simultaneously measure cytotoxic activity and cell cycle arrest, to identify the antiproliferative effect of IMiDs agents, such as lenalidomide (LEN), in plasma cells (PC) from whole bone marrow MM samples, as a putative secondary reflect of IMiDs activity, that could predict the clinical response ex vivo.

Material and Methods: Bone marrow samples from 16 MM patients were sent to Vivia from Polish Myeloma Consortium (PMC) and Spanish hospitals within 24h after extraction. Whole sample were incubated for 96h in well plates containing 8 concentrations of LEN. To induce plasma cell proliferation, PC were resuspended in IMDM medium supplemented with 20% autologous plasma. The number of proliferating and non proliferating live leukemic cells was determined using the CFDA dye, and the PC population and viability was determined labeling with monoclonal antibodies and Annexin V. Dose response curves of LEN for proliferating and non proliferating live pathological cells were measured, and pharmacological responses were calculated using pharmacokinetic population models.

Results: Preliminary results with a standard depletion assay do not allow patient stratification with IMIDs (Figure 2) that force us to explore new approaches. In this sense, we develop a new assay where IMIDs activity was explored in the myeloma cells that were induced to proliferate. As expected, a clear difference in the sensitivity of proliferating vs. non-proliferating MM cell subsets was observed. LEN was more active towards proliferative live tumor cells, with a median maximum effect (Emax) of 74% vs 48% and a 2 fold more sensitive potency (EC50) of 0.002 mM vs. 0.15 mM, concentrations achievable clinically. This effect was probably due to a cell cycle arrest (Figure 3 & 4). Interestingly, there is a great heterogeneity in the potency and effect (Figure 4) in all samples, pointing the possibility of patient selection for LEN treatment. In addition, the efficacy was incomplete in many patients (median Emax of proliferating live tumor cells equal to 48) leaving a significant number of proliferating cells, still even at the higher drug concentrations, that suggest the use of additional targets.

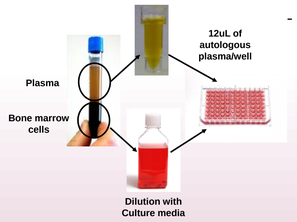
METHODS

- **Old procedure:**
  - Dilution of the whole sample with culture media:
    - Volume of sample per well depends on the cell counting
    - 12µl of FBS are added to each well to supplement the media
    - Final volume on each well is 60 µl of the mixture of the sample with culture media



- **Disadvantages:**
  - Physiologically, plasma represents 40% of the volume of the whole bone marrow sample
  - Percentage of plasma per well with this procedure is significantly reduced → **3%** of the volumen
  - Additional supplement, such as FBS, is required, varying the proportion of cytokines and other plasma components

- **New procedure:**
  - An initial centrifugation step of the whole sample is performed to separate the plasma



  - The cell fraction is diluted with culture media according to the following procedure:
    - **Volume of sample per well depends on the cell counting (between 1µl and 10µl)**
    - **12µl of plasma previously separated from the sample are added to each well**
    - **Final volume on each well → 60µl of the mixture of the sample with culture media.**
    - **No additional supplements (FBS) is required**

Percentage of plasma in this assay is 20%
More similar to physiological conditions

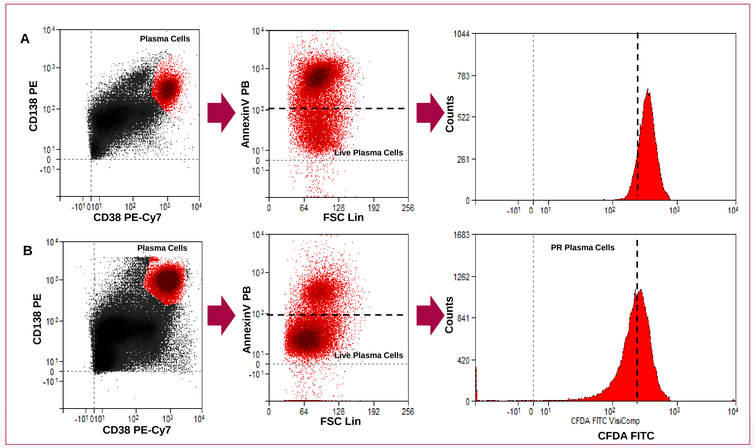


Figure 1. Gating strategy to identify the proliferating plasma cells. Cell surface markers and annexin-V are used to identify the live plasma cells population. CFDA staining is used to monitor plasma cell proliferation. Upper panel A represents a MM patient sample incubated without autologous plasma. The lower panels B shows a MM patient sample incubated with culture medium containing 20% autologous plasma. With this later approach, a higher viability and cells undergo proliferation can be observed.

RESULTS

Depletion assay

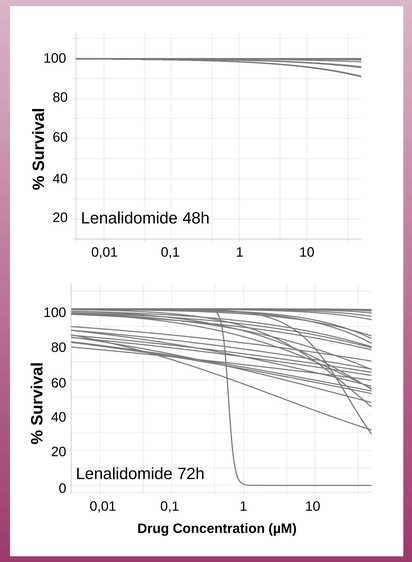


Figure 2. Dose response curves for LEN in 174 MM patient samples (upper panel at 48h) and 34 MM samples (lower panel at 72h) with the depletion assay measuring the cytotoxic activity of this drug.The grey lines display each individual response.

Proliferation assay

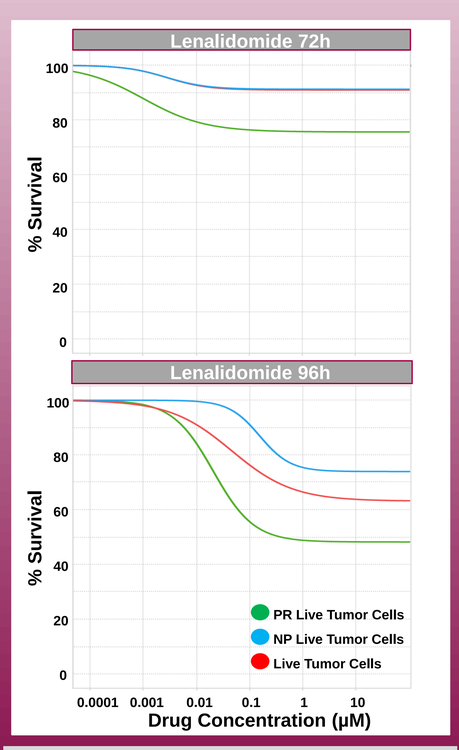


Figure 3. Median curves for proliferating (PR), non-proliferating (NP) and total Live Tumor Cells at 72h (upper panel) and 96h (lower panel). The optimal incubation time for the proliferation assay is established at 96h.

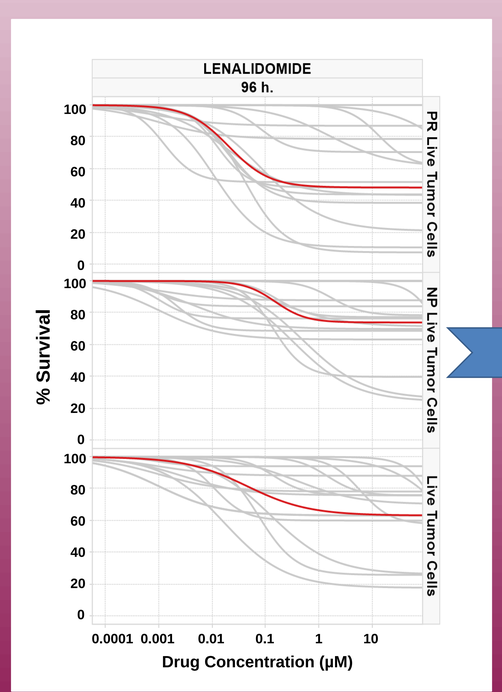


Figure 4. Dose response curves for LEN in 16 MM patient samples with the proliferation assay. The survival index (y-axis) ranges from 100% to 0% displaying the selective LEN effect in these patients calculated with PKPD population models.The grey lines display each individual response with the median response shown in red. PR: proliferating cells; NP: non-proliferating cells

	RESULT_TYPE	SAMPLE	EC50_µM	EMAX_Surv%	AUC
LENALIDOMIDE 96h	PR Live Tumor Cells	S1_001	0,0010	78,73	3938,04
		S1_002	0,0110	10,65	541,51
		S1_003	0,0173	43,92	2199,32
		S1_004	1,5899	61,33	3372,89
		S1_005	13,2547	61,60	3803,51
		S1_006	500,0000	29,99	4683,31
		S1_007	0,0386	7,51	392,31
		S1_008	0,0102	100,00	4999,99
		S1_009	0,0218	38,62	1936,91
		S1_010	0,0123	48,25	2414,01
		S1_011	0,0789	70,56	3533,19
		S1_012	ND	ND	ND
		S1_015	0,0012	51,67	2584,13
		S1_016	0,0827	20,96	1146,72
		S1_017	0,0204	43,49	2201,48
		S1_018	0,0010	87,00	4351,40
		Median	***0,0204***	***48,25***	***2584,13***
	NP Live Tumor Cells	S1_001	0,0181	100,00	4999,99
		S1_002	0,3216	24,25	1442,51
		S1_003	1,7573	78,48	4000,80
		S1_004	0,1965	71,44	3635,46
		S1_005	0,0010	76,49	3825,19
		S1_006	0,0044	69,84	3497,40
		S1_007	0,1399	39,77	2007,80
		S1_008	0,0010	84,12	4207,13
		S1_009	500,0000	100,00	4999,99
		S1_010	0,0022	68,68	3434,70
		S1_011	0,1638	77,33	3875,09
		S1_012	0,0010	63,23	3163,87
		S1_015	0,0010	87,86	4394,42
		S1_016	0,4964	25,91	1590,93
		S1_017	289,8307	0,00	4862,78
		S1_018	500,0000	100,00	4999,99
		Median	***0,1518***	***73,97***	***3850,14***
	Live Tumor Cells	S1_001	242,4046	0,00	4822,90
		S1_002	0,0165	17,76	921,82
		S1_003	1,4410	75,75	3859,27
		S1_004	0,3754	70,24	3611,57
		S1_005	5,9463	57,68	3320,66
		S1_006	0,0028	75,91	3798,63
		S1_007	0,0737	25,87	1312,85
		S1_008	0,0010	94,14	4707,81
		S1_009	0,0111	60,02	3002,13
		S1_010	0,0010	63,12	3158,25
		S1_011	0,1446	75,91	3803,60
		S1_012	0,0010	63,23	3163,87
		S1_015	0,0010	88,15	4408,45
		S1_016	0,1263	26,19	1431,51
		S1_017	0,0010	78,25	3913,89
		S1_018	500,0000	0,00	4547,64
		Median	***0,0451***	***63,17***	***3705,10***

Table 1. Estimation of the *ex vivo* population pharmacodynamic parameters in the 16 MM patient samples incubated with LEN at 96h. PR: proliferating cells; NP: non-proliferating cells

FUTURE PERSPECTIVES

❑ **Using the actual approach, the following studies are in process:**

- Correlate the expression of **Ikaros** (IKZF1) and **Aiolos** (IKZF3), essential for myeloma cell proliferation, with the capacity for these compounds to induce their **degradation** during the cell culture.
- Correlate the in vitro antiproliferative effects of the IMIDs with the **expression of CRBN, IRF4 and cyclin D1** before and after the cell culture.
- Explore **synergistic effects with other drugs** that target different pathways.

❑ **Under development to further explore the IMiDs activity:**

- Built representative **3D tissue scaffold** integrating **mesenchymal and osteoclast cells** to preserve primary multiple myeloma cells as a tool for **personalized therapeutics** with IMIDs where both antiproliferative and immunomodulatory effects are better represented.

CONCLUSIONS

❖ The incorporation of autologous serum enables the measurement of the effects of IMiDs on MM patients and thereby, a more accurately simulation of the in vivo conditions.

❖ This innovative assay offers better opportunities for *ex vivo* pharmacology in particular for the IMiDs, drugs with antiproliferative properties.

❖ The interpatient variability in the pharmacological profile for the LEN, if clinically validated, could help guiding a personalized treatment selection for IMiDs