Mechanisms Underlying the Synergistic Interaction of Filanesib with Pomalidomide and Dexamethasone (FPD) in Multiple Myeloma

Susana Hernández-García1, Laura San-Segundo1, Lorena González-Méndez1, Montserrat Martín-Sánchez1, Luis A Corchete1, Teresa Paino1, Ana Alicia López-Iglesias1, Esperanza M Algarín1, Mercedes Garayoa1, Brian J. Tunquist2, María Victoria Mateos1, Enrique M Ocio1

1 Hematology, University Hospital (IBSAL) & Cancer Research Center (IBMCC-CSIC), Salamanca, Spain; 2 Array BioPharma

Background and Aims
Filanesib (ARRY-520) is a novel inhibitor of the “kinesin spindle protein” (KSP), which has demonstrated efficacy in heavily pretreated patients with refractory MM, (Lonial et al, ASH 2013). Our preliminary studies demonstrated synergy with standard anti-MM agents, especially with pomalidomide and dexamethasone. This set the stage for a recently activated trial being run by the Spanish MM group investigating FPD in relapsed MM patients. In this work we investigate the mechanisms underlying the synergy of the combination.

Efficacy in vitro
FPD showed synergistic effect in MM cell lines.

Efficacy in vivo
FPD induced potent antmyeloma activity and increased the survival of treated mice.

Mechanism of action in vitro
Triple combination of FPD caused cell cycle arrest in G2/M phase and specific apoptosis of cells arrested in these proliferative phases in MM1S for 48 hours by Drq5 and Annexin V assay.

Results
In vitro action of FPD was evaluated in MM cell lines by MTT assay, bioluminescence, Annexin V staining, cell cycle analysis, and flow cytometry. Synergy was quantified with the CalcuSyn software. In vivo efficacy was assessed in a subcutaneous plasmacytoma model of MM1S in CB17-Scid mice. The mechanism of action was analyzed by Western blot, flow cytometry, genomic techniques, immunohistochemistry and immunofluorescence techniques.

Conclusions
Our results demonstrate that the synergy observed with filanesib in combination with pomalidomide and dexamethasone is the result of several coincidental mechanisms: a potentiation of the KSP inhibition with a subsequent increase in monopolar spindle formation and a simultaneous activation of the intrinsic and extrinsic pathways of apoptosis. In this regard, NOXA, BIM, BAX and BID are probably central players that, through different mechanisms, inhibit antagonistic proteins (MCL-1, BCL2 and BCL-XL) and promote mitochondrial outer membrane permeabilization and the release of apoptotic factors such as cytochrome C and AIF.