Poster # PB104

SKY-1214

A small molecule splicing modulator of members of the Fanconi anemia pathway for the treatment of multiple myeloma and non-Hodgkin's lymphoma

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Abstract

Background: Skyhawk Therapeutics uses a proprietary platform to discover small molecules designed to modulate RNA splicing upstream of protein production, focusing on biological targets once thought to be "undruggable." Here, we describe the preclinical characterization of *FANCL* and *FANCI* mRNA modulator SKY-1214, which shows an anti-tumor effect *in vitro* and *in vivo* in multiple myeloma (MM) and non-Hodgkin's lymphoma (NHL) models. *FANCL*/ *FANCI* are critical components of the Fanconi anemia DNA damage repair pathway, which is involved in the maintenance of genomic stability.

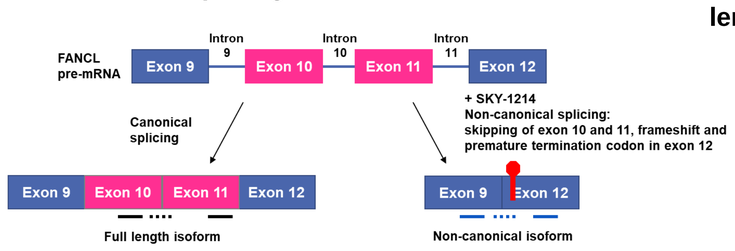
Material and methods: A panel of assays, including isoform-specific qPCR, RNA sequencing technologies, and cell viability assays, were applied to characterize SKY-1214's activity on splicing modulation and cell growth in MM and NHL cell models. The efficacy of SKY-1214 has been assessed *in vivo* by monitoring tumor growth inhibition in cell line-derived xenograft mouse models of the diseases.

Results: SKY-1214 modulates the splicing of *FANCL* and *FANCI* in a concentration-dependent manner in MM and NHL cell lines. This results in the decrease of the full length isoform and a parallel increase in the transcripts' non-canonical isoform through skipping exons 10/11 of *FANCL* and exon 32 of *FANCI*. SKY-1214 treatment for 3 to 7 days results in the reduction of proliferation and increase in apoptosis in MM and NHL cell lines, including models with high-risk cytogenetic and/or genetic alterations. A good correlation between splicing modulation of *FANCL* and viability is demonstrated in sensitive cellular models. *In vivo*, treatment with SKY-1214 results in tumor regression in xenograft mouse models (NHL Jeko-1 and MM KMS-28BM) at exposures that are well tolerated in mice.

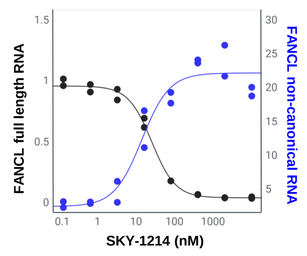
Conclusions: SKY-1214 is a splicing modulator of *FANCL* and *FANCI* that shows anti-cancer activity *in vitro* and *in vivo* in MM and NHL models. The new mechanism of action of this oral small molecule, together with its excellent bioavailability and pharmacologic properties, warrants future development in the clinic.

SKY-1214 is a splicing modulator of *FANCL* and *FANCI* in the Fanconi anemia DNA repair pathway, identified using the SKYSTAR® platform

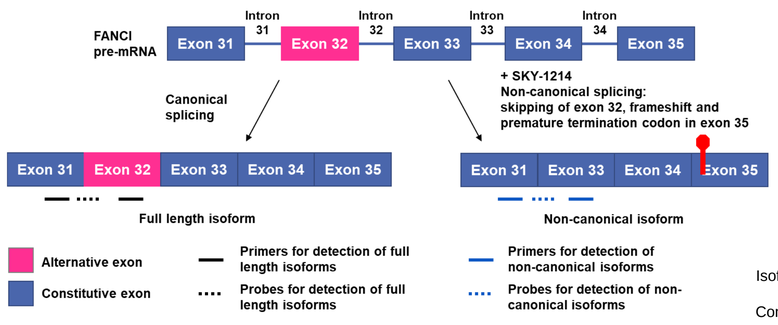
SKY-1214 splicing modulation of *FANCL*

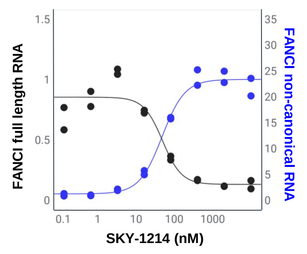


SKY-1214 potently reduces the full length mRNA of *FANCL/I* in MM cells



SKY-1214 splicing modulation of *FANCI*

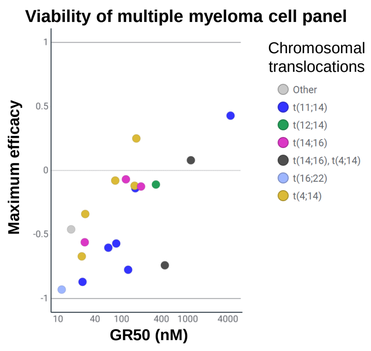


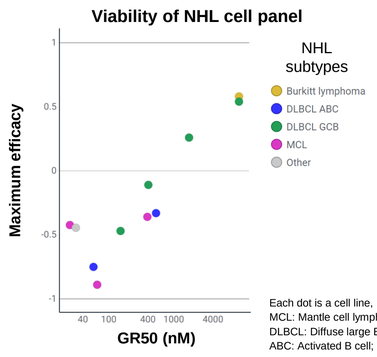


Isoform-specific qPCR analysis of full length and non-canonical mRNAs (fold change to DMSO) in KMS-28BM cells. Comparable results are obtained in other MM and NHL models.

SKY-1214 is a small molecule with anti-cancer activity in multiple myeloma and non-Hodgkin's lymphoma cell models

SKY-1214 potently reduces cell growth and induces cell death in MM cells with different chromosomal alterations and NHL cells from different subtypes

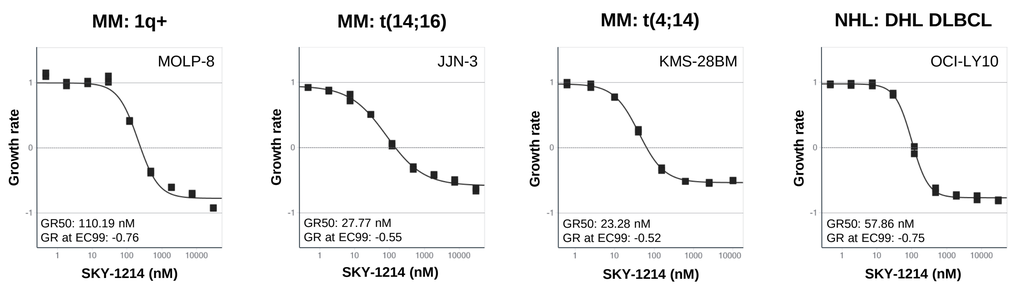




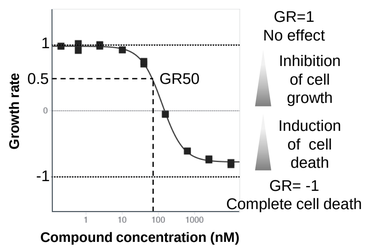
SKY-1214 potently (GR50 in low nM range) induces cell death (GR<0) of multiple myeloma and non-Hodgkin's lymphoma models with diverse cytogenetic alterations and from different subtypes

Each dot is a cell line,
MCL: Mantle cell lymphoma;
DLBCL: Diffuse large B cell lymphoma;
ABC: Activated B cell; GCB: Germinal Center B cell

SKY-1214 induces cell death in MM and NHL cell models with high-risk alterations



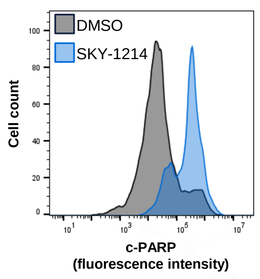
Parameters for the characterization of the effect of SKY-1214 on cell growth and viability



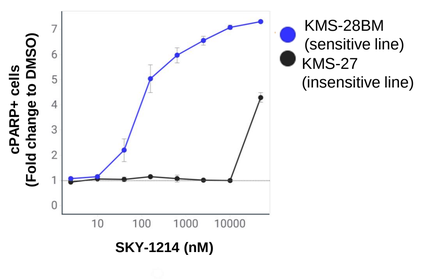
SKY-1214 potently induces cell death in models of MM with high-risk cytogenetic alterations 1q+, t(14;16), t(4;14) and others. In NHL, SKY-1214 is highly efficacious in models of high-risk double hit lymphoma (DHL)

SKY-1214 induces apoptosis in sensitive multiple myeloma cell lines

SKY-1214 treatment increases the levels of the apoptotic marker c-PARP in sensitive MM cell line KMS-28BM

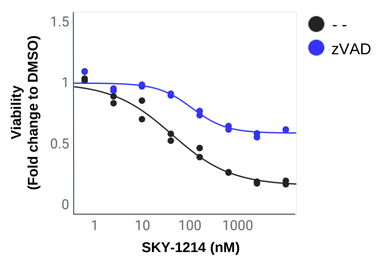


c-PARP levels measured by flow cytometry in KMS-28BM cells treated with DMSO or 156 nM SKY-1214 for 48hr



Quantification of c-PARP induction in sensitive KMS-28BM and insensitive KMS-27 cells upon 48hr treatment with SKY-1214

Inhibition of caspases protects KMS-28BM cells from the anti-cancer activity of SKY-1214

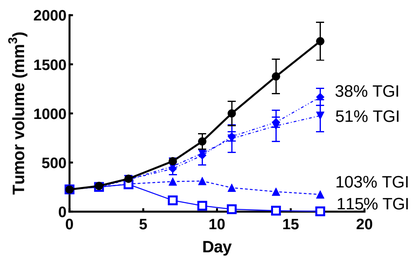


Cell viability in response to SKY-1214 alone or in presence of the pan-caspase inhibitor zVAD

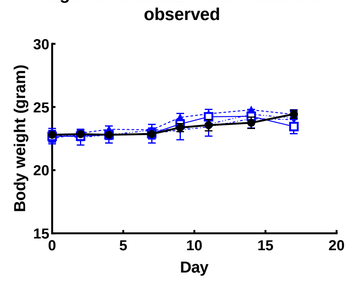
SKY-1214 is orally bioavailable and induces tumor growth inhibition and regression in multiple myeloma and non-Hodgkin's lymphoma xenograft mouse models

SKY-1214 exerts antitumor activity as single agent *in vivo* in the MM KMS-28BM xenograft mouse model

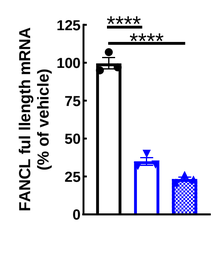
Dose-dependent tumor growth inhibition and regression in KMS-28BM CDX mice

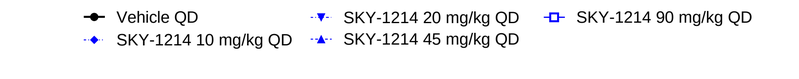


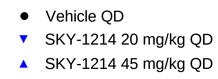
SKY-1214 treatment was well tolerated at all doses and no changes in body weight of KMS-28BM CDX mice were observed



***FANCL* full length mRNA is reduced in tumors from SKY-1214 treated mice**





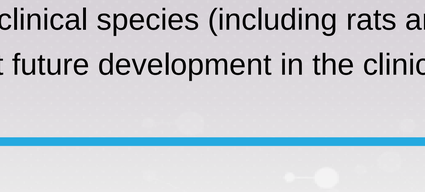


NOD SCID mice were inoculated subcutaneously with KMS-28BM cells for tumor development. SKY-1214 was administered orally once a day for 18 days. For *FANCL* analysis, mRNA was extracted from tumors and isoform-specific qPCR was performed. qPCR analysis was not performed in the 90 mg/kg group because the tumors were undetectable in the majority of the animals. Data are presented as mean ± SEM. One way ANOVA, ****P<0.0001. TGI: tumor growth inhibition

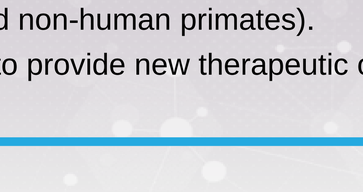
- All KMS-28BM CDX mice in the 45 mg/kg and 90 mg/kg groups demonstrated progressive tumor regression until the scheduled study termination. Furthermore, 6 out of 7 mice from the group treated with 90 mg/kg dose showed complete tumor eradication as assessed by caliper.

SKY-1214 exerts antitumor activity as single agent *in vivo* in the NHL Jeko-1 xenograft mouse model

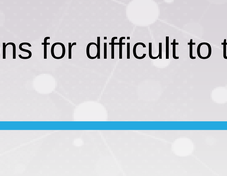
Dose-dependent tumor growth inhibition and regression in Jeko-1 CDX mice



SKY-1214 treatment was well tolerated at all doses and no changes in body weight of Jeko-1 CDX mice were observed



***FANCL* full length mRNA is reduced in tumors from SKY-1214 treated mice**



Balb/c nude mice were inoculated subcutaneously with Jeko-1 cells for tumor development. SKY-1214 was administered orally once a day for 18 days. For *FANCL* analysis, mRNA was extracted from tumors and isoform-specific qPCR was performed. qPCR analysis was not performed in the 45 and 90 mg/kg group because tumors were of limited size or undetectable. Data are presented as mean ± SEM. Unpaired t-test, ***P<0.001. TGI: tumor growth inhibition

- All Jeko-1 CDX mice from groups treated with 45 and 90 mg/kg doses showed progressive tumor regression until the end of the study. All mice in the 90 mg/kg group and 3 out of 7 mice in the 45 mg/kg group demonstrated complete tumor eradication as assessed by caliper.
- Complete tumor regression achieved in mice treated with the highest dose of SKY-1214 was sustained for 8 days after treatment cessation (on day 10) until the study termination (day 18).

Conclusion

- SKY-1214 is a novel, orally bioavailable small molecule splicing modulator. SKY-1214 shows potent and efficacious anti-cancer activity both *in vitro* and *in vivo* in models of multiple myeloma and non-Hodgkin's lymphoma, including models of high-risk disease. *In vivo*, SKY-1214 achieves tumor growth inhibition and tumor regression to undetectable levels at tolerated doses in mice.
- SKY-1214 has completed pharmacokinetic characterization and tolerability assessment in preclinical species (including rats and non-human primates).
- The novel mechanism of action, efficacy and pharmacological properties of SKY-1214 warrant future development in the clinic to provide new therapeutic options for difficult to treat multiple myeloma and non-Hodgkin's lymphoma.