

SKY-1214

A Small Molecule Splicing Modulator Targeting FANCL and FANCI, Provides a New Mechanism of Action Targeting Multiple Myeloma and Non-Hodgkin's Lymphoma

S. Rauch¹, S. Reber¹, M. Perez Salvia¹, M. Pregnotato¹, B. Liu², L. Berry², O. Buiakova², H. Ratni¹, B. Guden², C. Cusulin¹, L. Shanahan¹, D.V. Faller¹, V. Costa¹, S. Paushkin²

¹Skyhawk Therapeutics, Hochbergerstrasse 60i, 4058 Basel, CH; ²Skyhawk Therapeutics, Inc. 180 3rd Ave., Waltham, MA 02451, USA

Abstract

Introduction: The increasing number of elderly and fragile patients with relapsed/refractory (R/R) Multiple Myeloma (MM) and Large B-cell Lymphoma (LBCL) after chemotherapy and immunotherapies necessitates new, effective and convenient therapies. Novel mechanisms of action are required to overcome the resistance generated by prior treatments. The understanding of RNA biology and the capacity to develop oral drugs for “undruggable” protein targets opens a new opportunity to address these treatment needs.

FANCL and *FANCI* are critical components of the Fanconi anemia DNA damage repair pathway, involved in the maintenance of genome integrity. SKY-1214 is a small molecule that modulates splicing of *FANCL* and *FANCI* pre-mRNA, causing exon skipping and introduction of premature termination codons leading to the degradation of the *FANCL* and *FANCI* mRNAs. Preclinical characterization of SKY-1214 showed monotherapy activity in multiple MM and NHL cell lines and in cell-derived xenograft (CDX) models, including models of high-risk MM and NHL. We describe here the results of *in vitro* and *in vivo* studies of SKY-1214 in combination with agents commonly used for the treatment of R/R MM and R/R NHL.

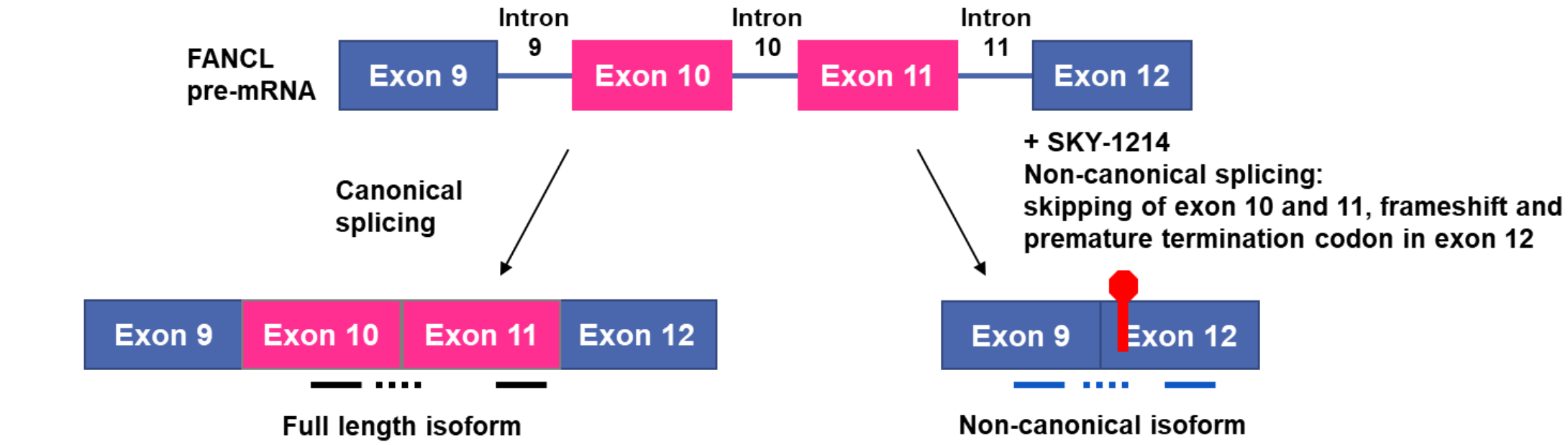
Methods: Cell growth and viability were assessed using CellTiter-Glo2.0 assay. The SynergyFinder Rpackage was used to calculate synergy scores for the combination treatments *in vitro*. CDX MM and NHL models were used to study combination treatments in which tumor growth and body weight were monitored.

Results: Treatment with SKY-1214 in combination with dexamethasone (for MM), vincristine (for NHL) and selinexor (for MM and NHL) resulted in improved inhibition of cell growth and viability in multiple cell lines compared to the effect of SKY-1214 alone. Bliss synergy scores indicative of additivity were obtained for all the combinations and models tested. Cell lines resistant to dexamethasone and bortezomib remained as responsive to SKY-1214 as the parental non-resistant cell lines. *In vivo* studies of SKY-1214 in combination with selinexor, vincristine and dexamethasone showed improved activity compared to monotherapy treatments in MM and/or NHL CDX models. Combination with dexamethasone resulted in enhanced durability of effect upon treatment cessation. All *in vivo* combinations showed acceptable tolerability as determined by stable body weight measurements throughout the studies.

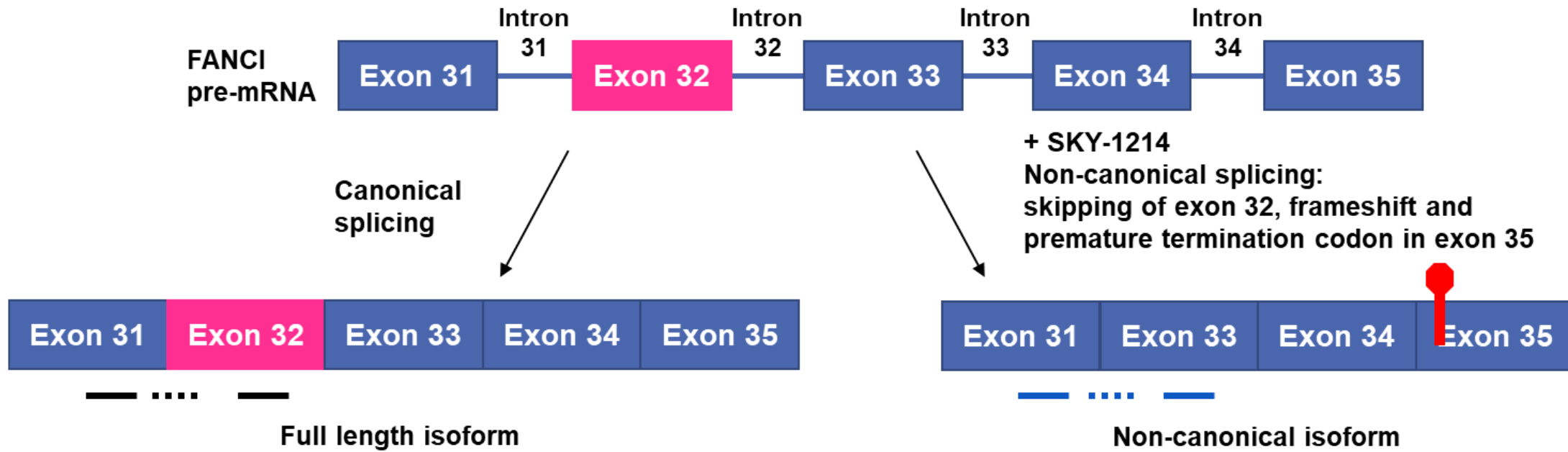
Conclusions: SKY-1214 treatment causes tumor cell growth inhibition and apoptosis in a broad panel of MM and NHL lines. Tumor regressions were observed in CDX models of NHL and high-risk MM treated with SKY-1214, both as monotherapy and in combination. These data, together with the favorable oral bioavailability and pharmacologic characteristics of SKY-1214 with a distinctive mechanism of action, warrant future clinical development to address the unmet medical need of the growing multi-refractory MM and NHL patient populations.

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

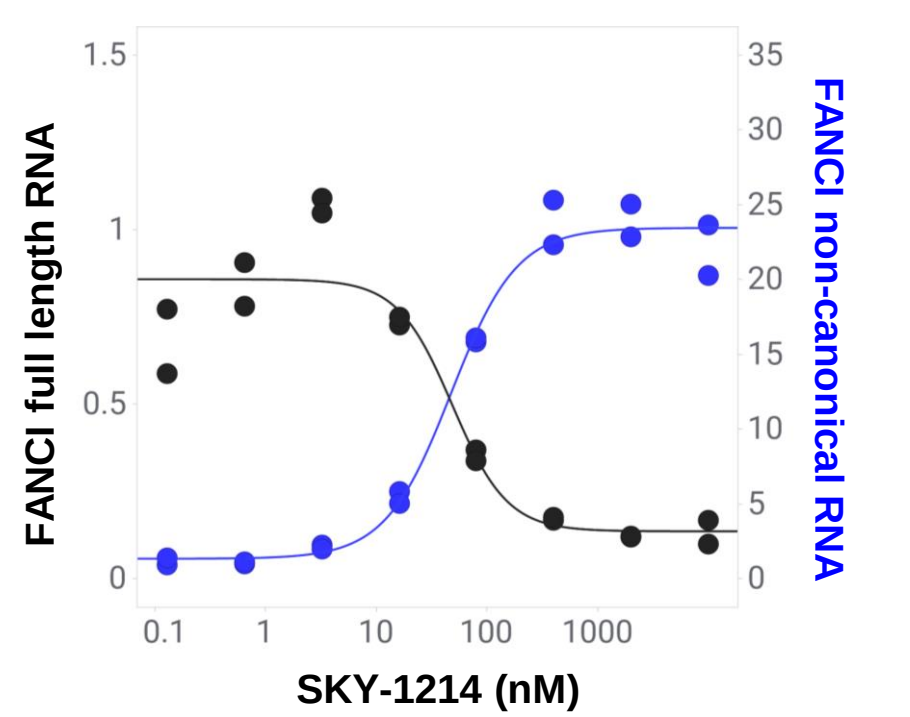
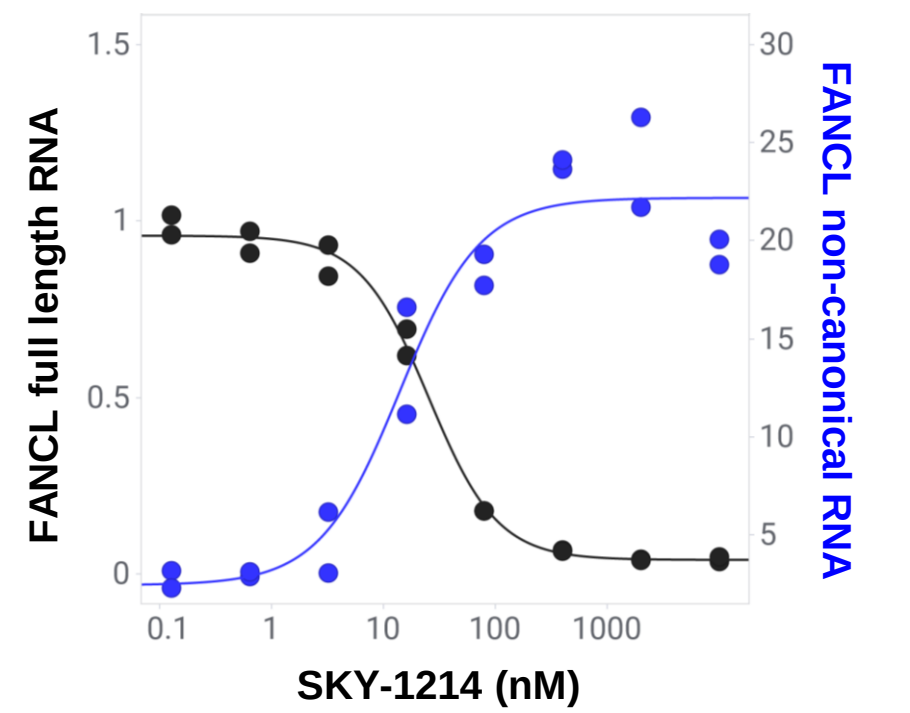


SKY-1214 splicing modulation of FANCI



Alternative exon
Constitutive exon
Primers for detection of full length isoforms
Probes for detection of full length isoforms
Primers for detection of non-canonical isoforms
Probes for detection of non-canonical isoforms

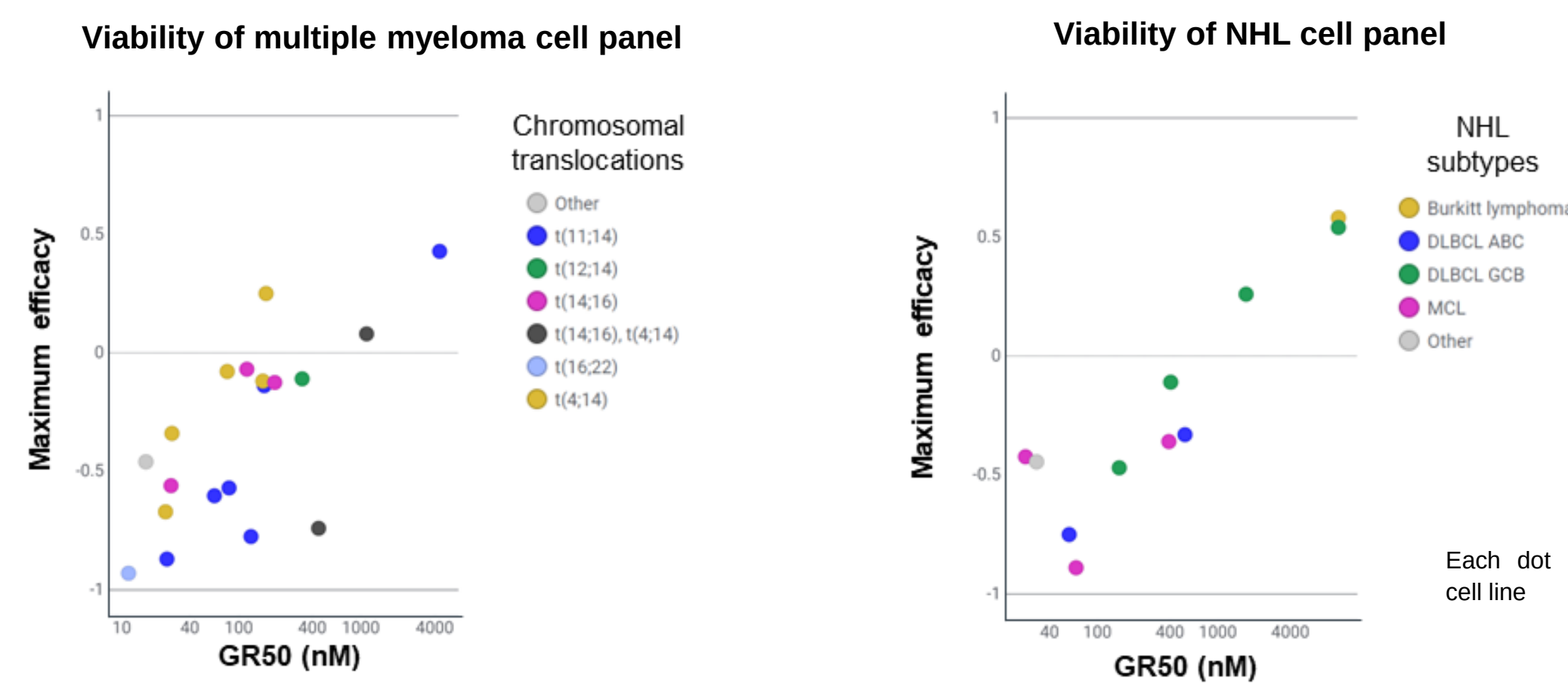
SKY-1214 potently reduces the full length mRNA of FANCL/I in MM cells



Isomform-specific qPCR analysis of full length and non-canonical mRNAs in KMS-28BM cells. Comparable results are obtained in other MM and NHL models.

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

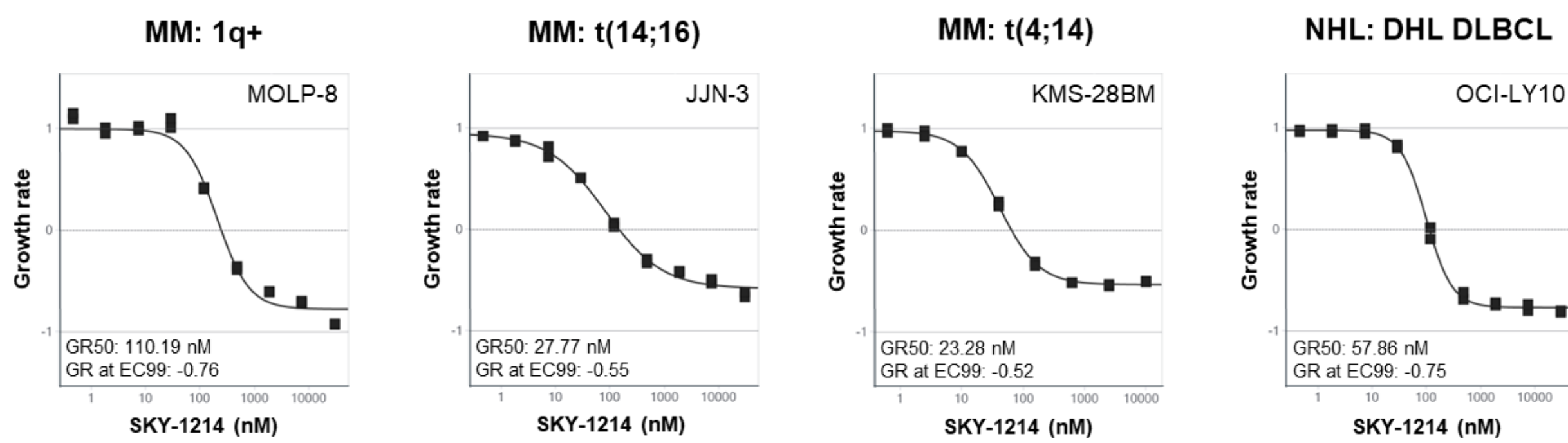
SKY-1214 potently reduces cell growth and induces cell death in MM cells with different chromosomal alterations and NHL cells from different subtypes



SKY-1214 potently (GR50 in low nM range) reduces cell viability (Maximum efficacy or GRmax<0) of multiple myeloma and non-Hodgkin's lymphoma models with diverse characteristics. SKY-1214 induces apoptosis in sensitive lines (*not shown*)

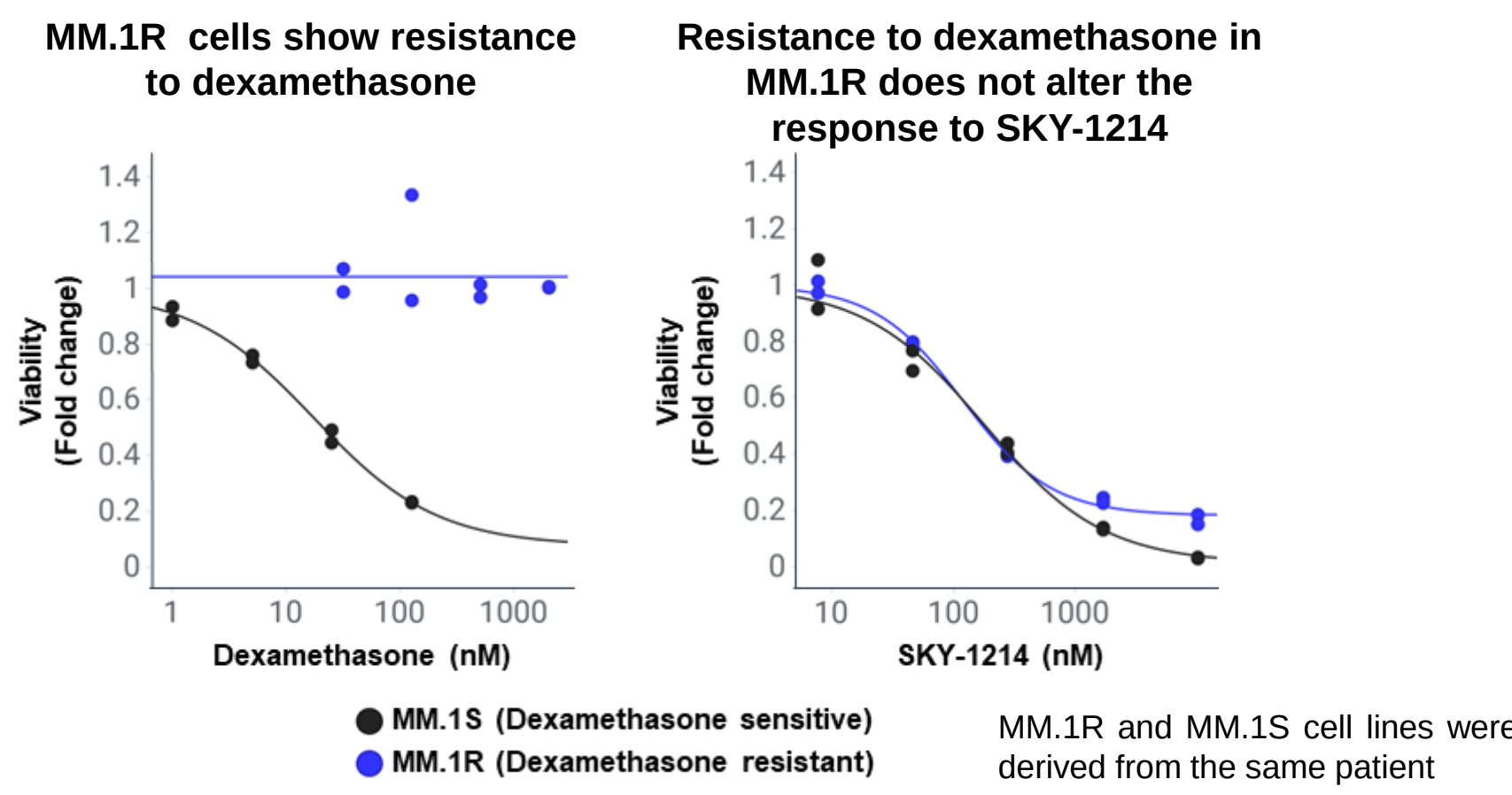
GR: growth rate. GR=1 indicates no changes in cell growth. GR=0 indicates cytostatic effect. GR<0 indicates cytotoxic effect. Maximum efficacy refers the maximum effect measured as GR (GRmax)

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



SKY-1214 potently reduces cell viability in models of MM with the 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)

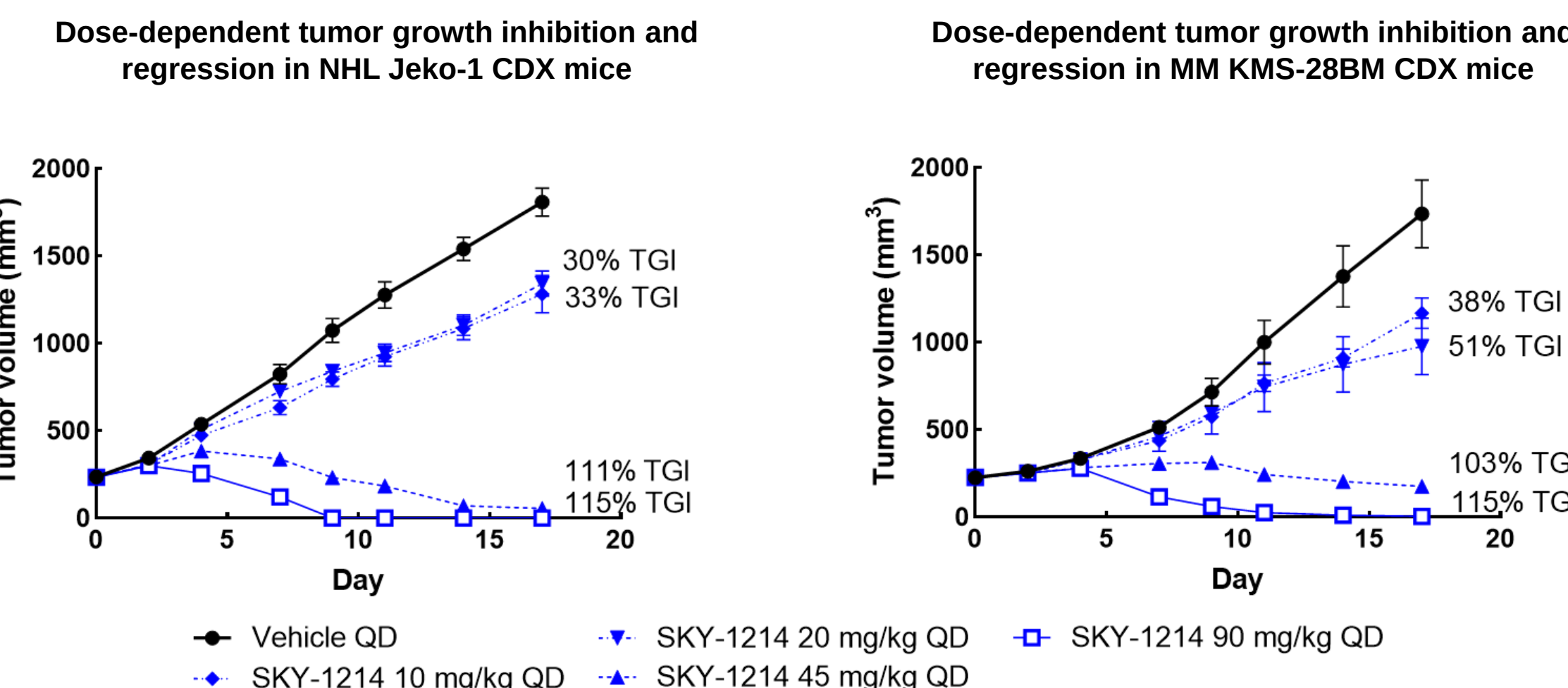
SKY-1214 shows comparable anti-cancer activity in a cell line pair sensitive or resistant to dexamethasone



SKY-1214 treatment resulted in comparable anti-cancer effect in MM.1R and MM.1S cell lines, which are dexamethasone-resistant and sensitive, respectively. The activity of SKY-1214 appears not to be affected by the acquisition of resistance to dexamethasone.

Similar results were obtained in a cell line pair sensitive or resistant to bortezomib.

SKY-1214 exerts antitumor activity as single agent *in vivo* in the MM KMS-28BM and the NHL Jeko-1 xenograft mouse models



Balb/c nude and NOD SCID mice were inoculated subcutaneously with Jeko-1 and KMS-28BM cells, respectively. 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. Data are presented as mean ± SEM. TGI: tumor growth inhibition

All Jeko-1 and KMS-28BM CDX mice in the 45 mg/kg and 90 mg/kg groups demonstrated progressive tumor regression until the study termination.

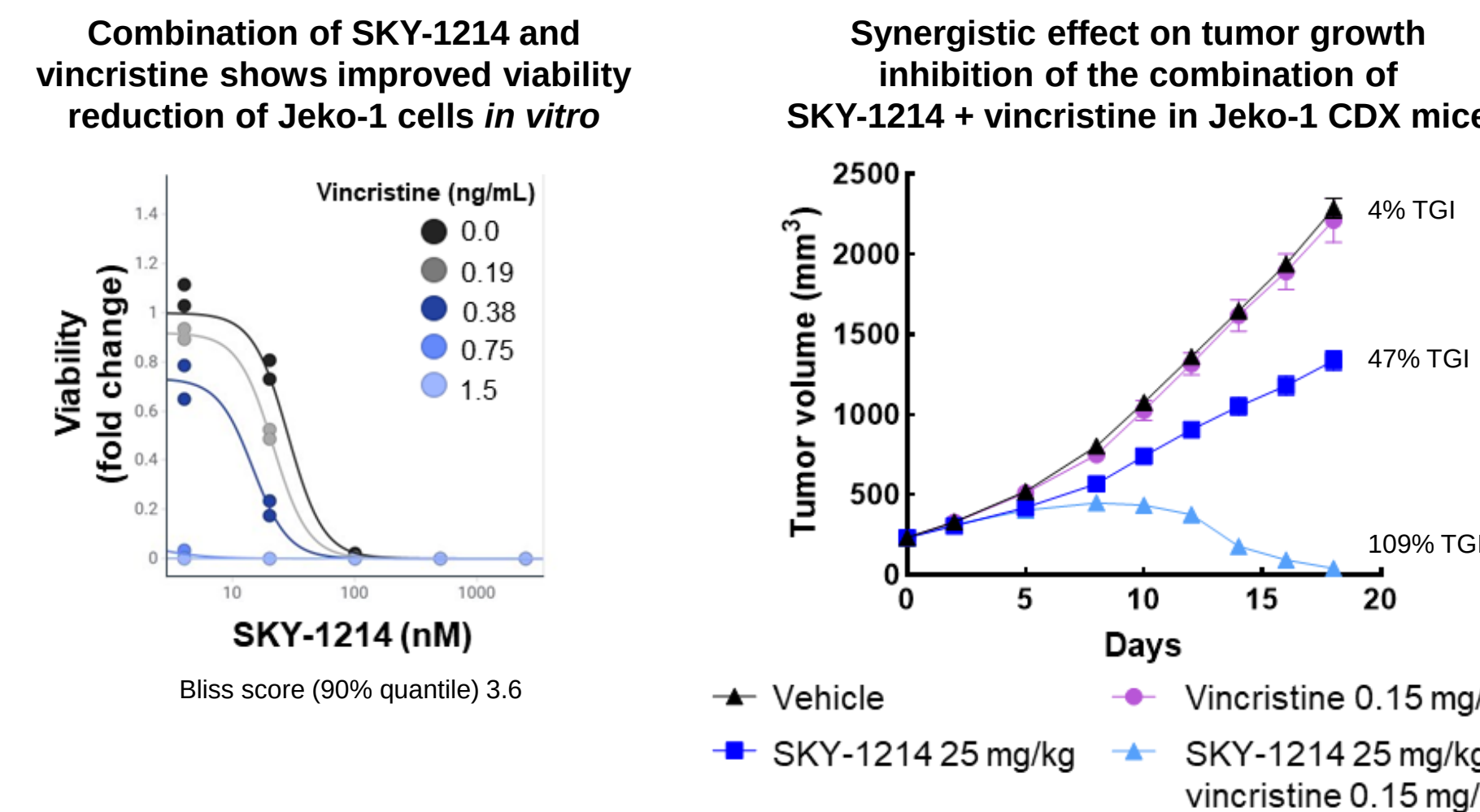
The treatment resulted in complete tumor regression at the highest doses (KMS-28BM: 6/7 mice at 90 mg/kg; Jeko-1: 3/7 mice at 45 mg/kg and all mice at 90 mg/kg).

In Jeko-1, complete tumor regression at the highest dose was sustained for 8 days after treatment cessation (on day 10) until the study termination (day 18).

SKY-1214 treatment was well tolerated at all doses (no body weight loss) and *FANCL* mRNA was reduced in tumors from treated mice (*not shown*)

Combination of SKY-1214 with small molecule agents used for the treatment of multiple myeloma and non-Hodgkin's lymphoma results in improved anti-cancer activity

SKY-1214 and vincristine combination in NHL



Jeko-1 CDX mice were treated with suboptimal doses of SKY-1214 and vincristine as single agents or in combination.

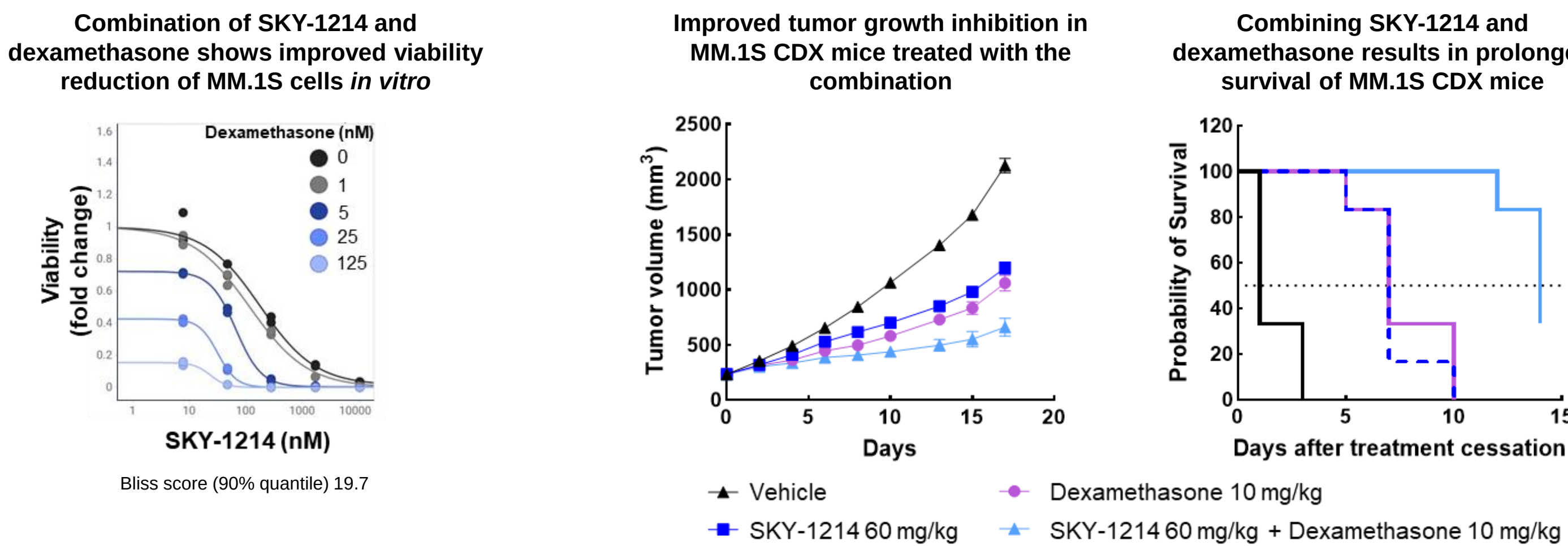
The combination of SKY-1214 and vincristine exceeded the effect of each compound as single agent *in vitro* and *in vivo*.

In vivo, the combination increased the durability of the effect after treatment cessation (*not shown*).

No adverse effects of the treatments were observed in the Jeko-1 CDX mice (no body weight loss)

Balb/c nude mice were inoculated subcutaneously with Jeko-1 cells. SKY-1214 was administered orally once a day for 21 days. Vincristine was administered once a week intravenously. Data are presented as mean ± SEM. TGI: tumor growth inhibition

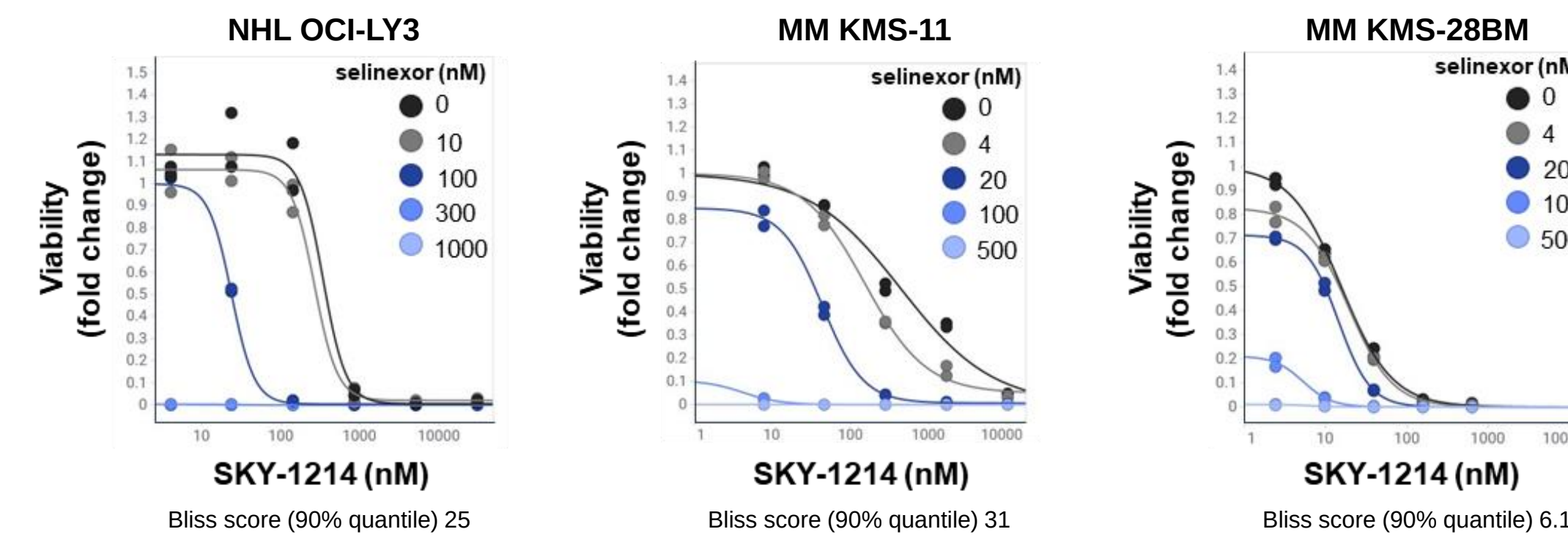
SKY-1214 and dexamethasone combination in MM



NOD SCID mice were inoculated subcutaneously with MM.1S cells. MM.1S CDX mice were treated with suboptimal doses of SKY-1214 and dexamethasone. SKY-1214 was administered orally once a day for 18 days. Dexamethasone was administered twice a week intraperitoneally. Data are presented as mean ± SEM. After treatment cessation, the effect of treatments on survival time to humane study endpoint was monitored.

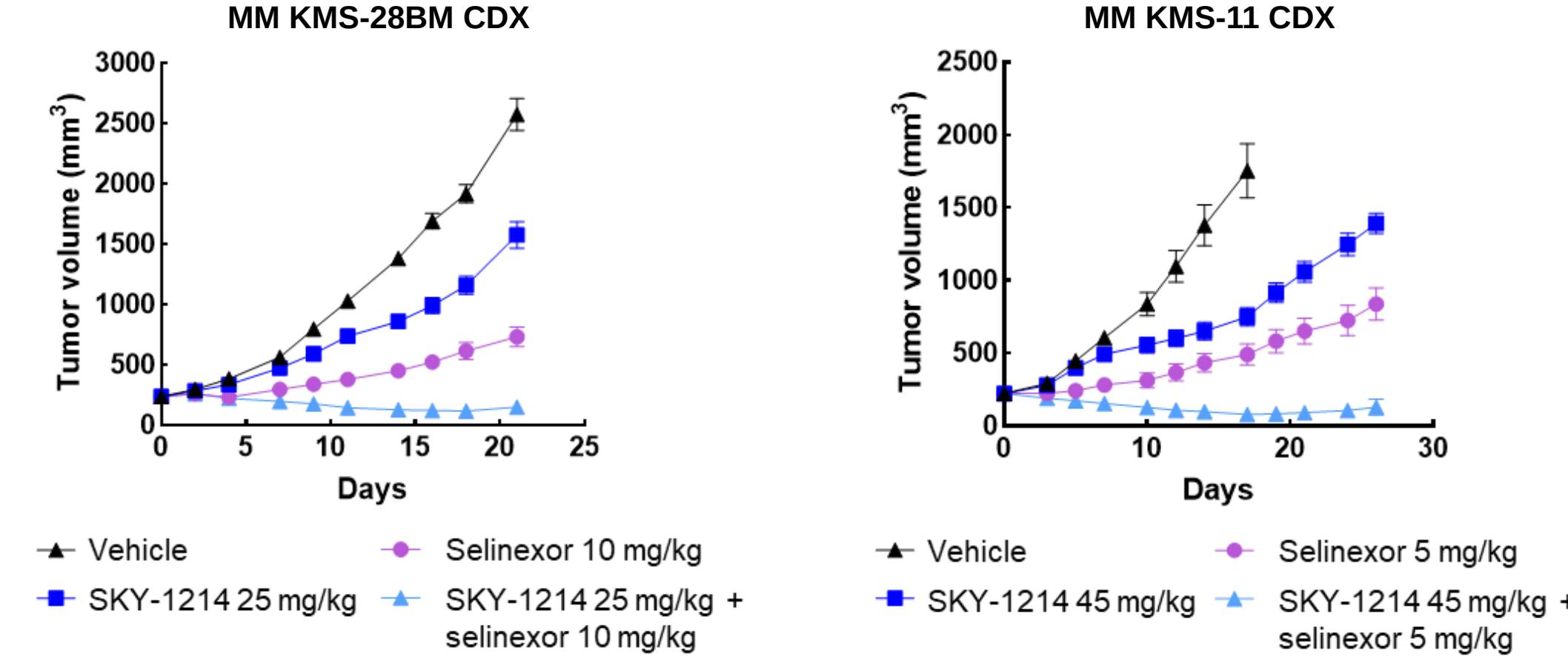
SKY-1214 and selinexor combination in MM and NHL

Combination of SKY-1214 and selinexor shows improved viability reduction of multiple MM and NHL cell lines *in vitro*



The combination of SKY-1214 and selinexor resulted in additive and synergistic effect in a panel of MM and NHL cell lines *in vitro*. Shown are selected examples

Improved tumor growth inhibition *in vivo* upon treatment with SKY-1214 combined with selinexor



SKY-1214 and selinexor were administered at suboptimal doses resulting in tumor growth inhibition in two MM CDX models

The combination of SKY-1214 and selinexor resulted in consistent and progressive tumor regression in KMS-11 and KMS-28BM CDX mice, exceeding the effect of the single agents

NOD SCID mice were inoculated subcutaneously with KMS-28BM or KMS-11 cells. Mice were treated with suboptimal doses of SKY-1214 and Selinexor. SKY-1214 was administered orally once a day for 22 (KMS-28BM) or 29 (KMS-11) days. Selinexor was administered orally twice a week. Data are presented as mean ± SEM.