

ALRN-6924, a Dual Inhibitor of MDMX and MDM2 that Causes Minimal Thrombocytopenia in Patients, Disrupts Different Stages of Thrombopoiesis Compared to MDM2-only Inhibition



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Abstract

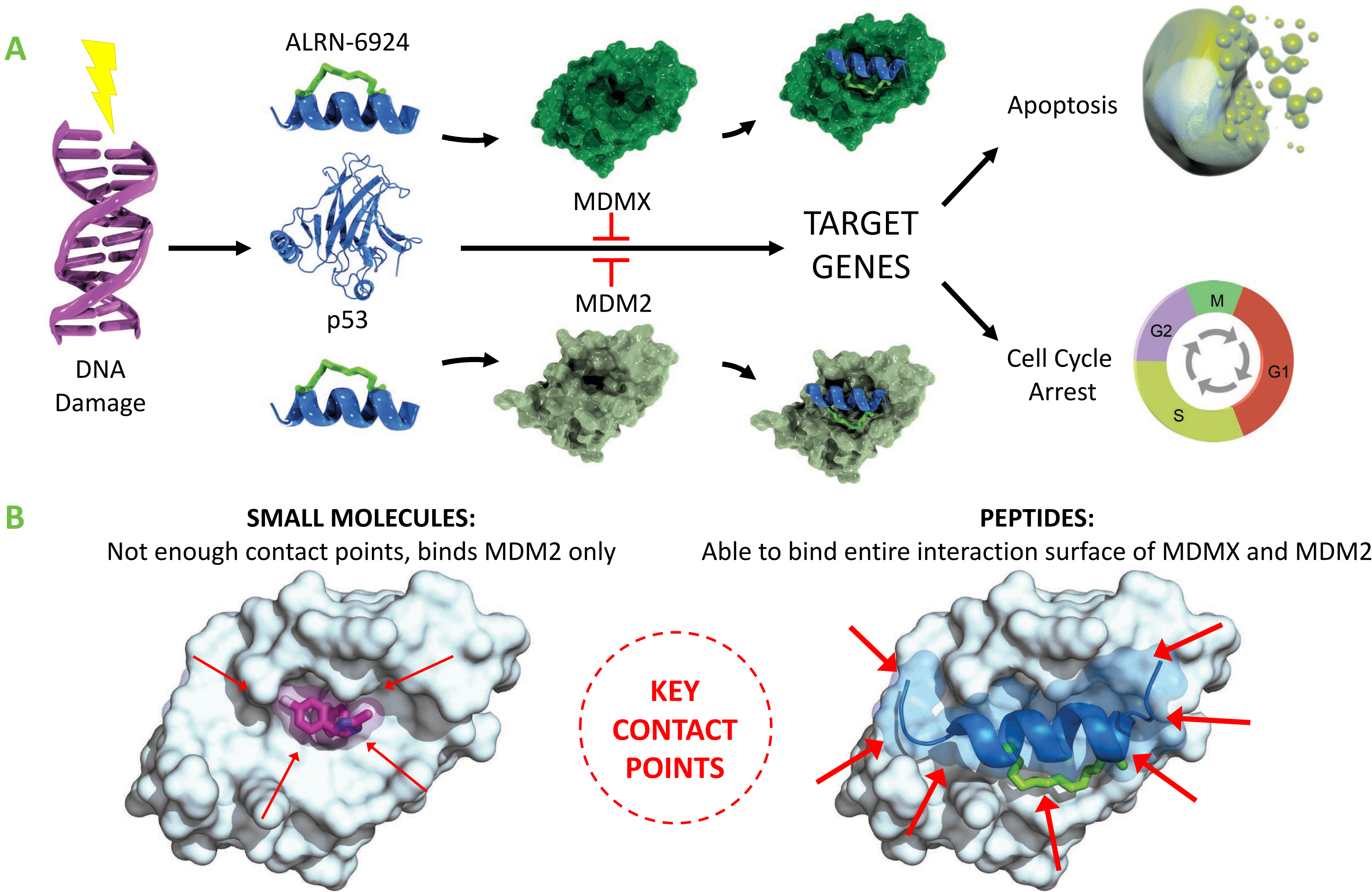
Background: ALRN-6924 is a stabilized, cell-permeating α -helical peptide that disrupts interactions of the p53 tumor suppressor protein with its endogenous inhibitors, MDMX and MDM2. ALRN-6924 can thereby restore p53-dependent anti-cancer activity in p53 wild type tumors. In an ongoing ALRN-6924 clinical trial with solid tumor and lymphoma patients enrolled as of 13 August, 2018 there have been 1/0 adverse events (AE) of Grade 3/4 thrombocytopenia (TCP), respectively, at doses that yield objective responses (NCT02264613)^{1,2,3}. In contrast, small molecule MDM2-only inhibitors induce Grade 3-4 TCP as a common AE and dose-limiting toxicity.

Aim: To explore the differences in TCP grades and frequencies from published solid tumor trials for ALRN-6924 and MDM2i's, we investigated the effects of dual MDMX/MDM2-inhibition vs. MDM2-only inhibition during different stages of megakaryocyte maturation.

Methods: Clonogenicity was measured in megakaryocyte (MK), erythroid (E), and granulocyte/monocyte myeloid (GM) progenitor colony-forming unit (CFU) assays. Human bone marrow CD34+ cells were seeded in methylcellulose semi-solid media containing IL-3 and IL-6; and TPO, Epo or GM-CSF together with ALRN-6924, RG7388 (idasanutlin), or controls. CFU-MK, CFU-E, and CFU-GM colonies were measured after 14 days of culture. Megakaryocyte maturation was modeled *in vitro* using a 14-day liquid culture assay. Human bone marrow CD34+ cells were cultured in liquid medium containing SCF and TPO. Megakaryocyte maturation was assessed by measuring the expression of CD41 and CD42. To assess the effects of ALRN-6924 and RG7388 on platelet development, drugs were added to the culture at different stages during the 14-day period of megakaryocyte maturation *in vitro*. DNA synthesis was measured by BrdU, and apoptosis by measuring Annexin V and propidium iodide staining. Healthy C57BL/6 mice treated with ALRN-6924 3x/week were monitored for blood counts 2x/week; hematopoietic stem and progenitor cells in bone marrow were measured at 4 weeks.

Results: *In vitro*, ALRN-6924 disrupted MK colony formation more potently than RG7388, with IC₅₀ of 0.024 and 0.12 μ M, respectively, while their effects were comparable in CFU-E and CFU-GM assays with IC₅₀ for both of $\approx$ 0.2 μ M. Conversely, RG7388 disrupted megakaryopoiesis by inducing apoptosis and cell cycle arrest in maturing MKs 10-fold more potently than ALRN-6924. Consistent with these findings, ALRN-6924 induced minimal effects on platelet counts in mice at doses that are effective in xenotransplanted and syngeneic tumor models, despite a significant decrease in MK and erythroid progenitors, while more immature hematopoietic stem and progenitor cells numbers remained unperturbed.

Figure 1: The Stabilized, Cell-Permeating α -Helical Peptide ALRN-6924 is a First-in-Class Dual Inhibitor of MDMX and MDM2



A) Mechanism of action of ALRN-6924. The tumor suppressor p53 is one of the most pursued targets in oncology, playing a central role inducing cell cycle arrest, apoptosis, senescence, autophagy, cellular metabolism and immune surveillance in response to cellular stresses such as DNA damage and oncogenic signals⁴. ALRN-6924 is a stabilized p53 peptide, dual MDMX/MDM2 inhibitor that has demonstrated anticancer activity as monotherapy in clinical trials^{1,2,3}. **B)** Stabilized peptides mimic natural peptide sequences at the interface of protein-protein interactions, displaying a larger surface area of interaction with its target, and providing superior binding properties which reduce off-target effects and the risk of acquiring mutations associated with resistance. Furthermore, a single compound can engage with ≥ 2 targets, e.g. MDMX + MDM2.

Table 1: MDM2i Drugs: Phase 1 All-comers Trials

Drug	First-in-human Phase 1	# pts	% pts w/ related Thrombocytopenia AEs, G $\geq$ 3	% pts w/ related Neutropenia AEs, G $\geq$ 3
ALRN-6924	All-comers solid tumors and lymphomas	96	1%	3%
AMG 232 ⁵	All-comers solid tumors	39	33%	21%
DS-3032b ^{6,7}	All-comers solid tumors and lymphomas	103	19%	12%
HDM201 ^{8,9}	All-comers solid tumors	107	24%	23%
RO6839921 ¹⁰	All-comers solid tumors	41	15%	20%
RG7388 ¹¹	All-comers solid tumors and lymphomas	95	33%	21%
MK-8242 ¹²	All-comers solid tumors	47	15%	19%

Figure 2: Ex vivo Megakaryocyte Development Assay

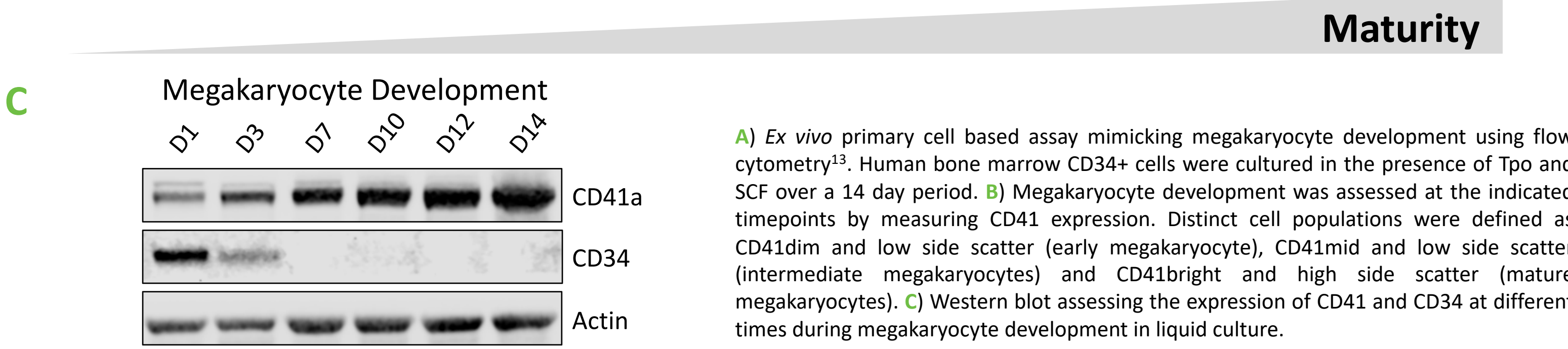
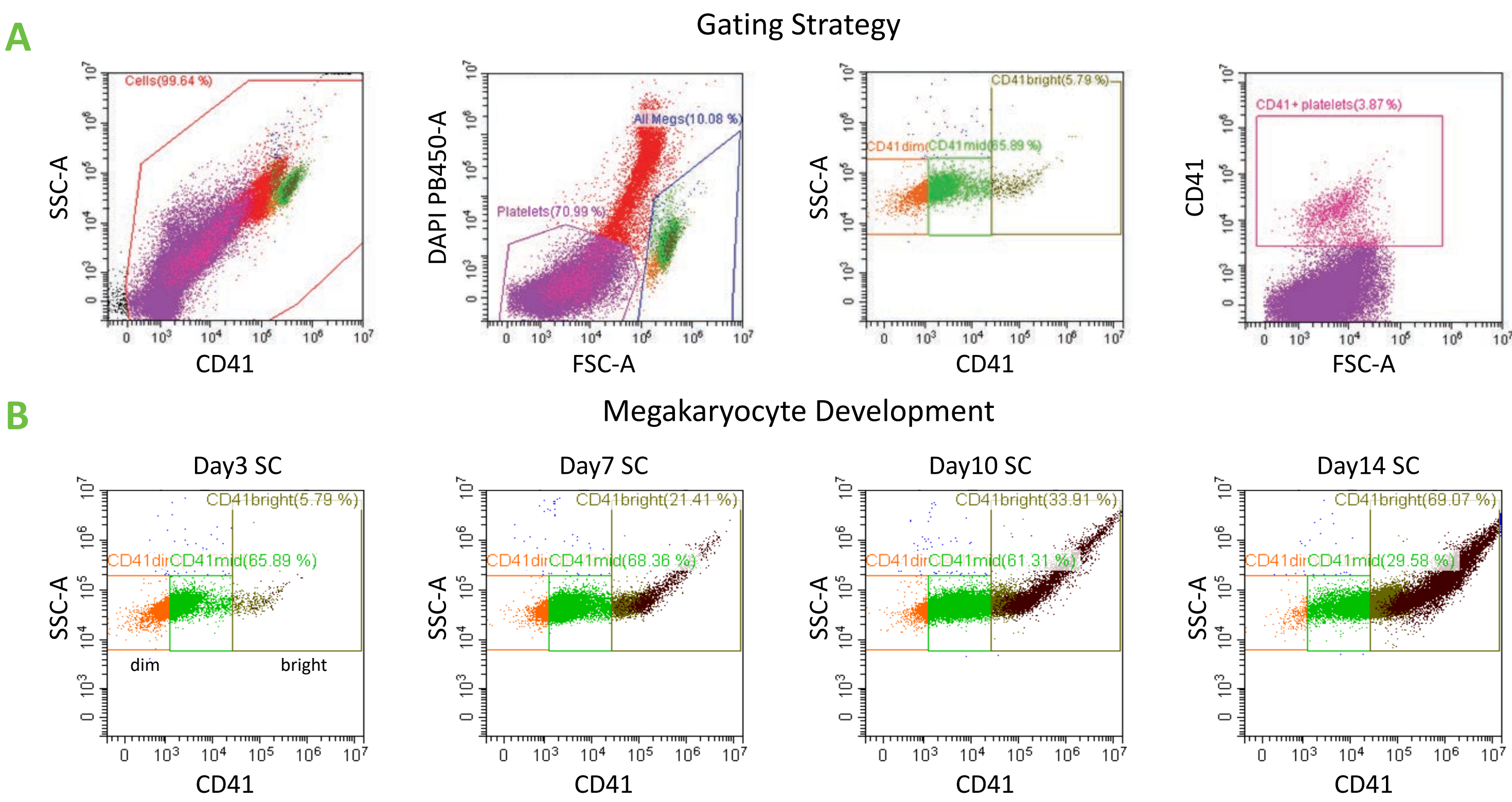
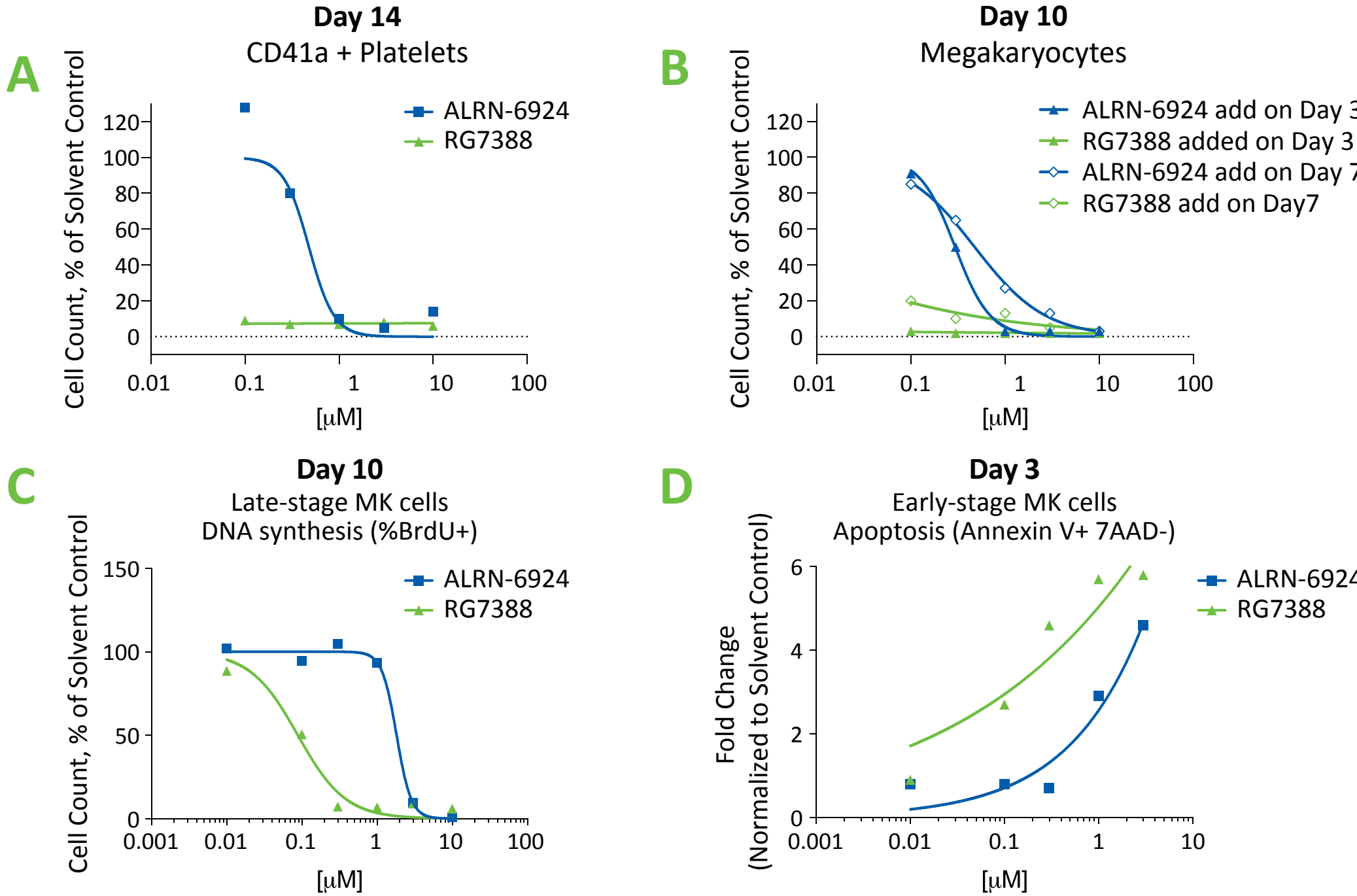
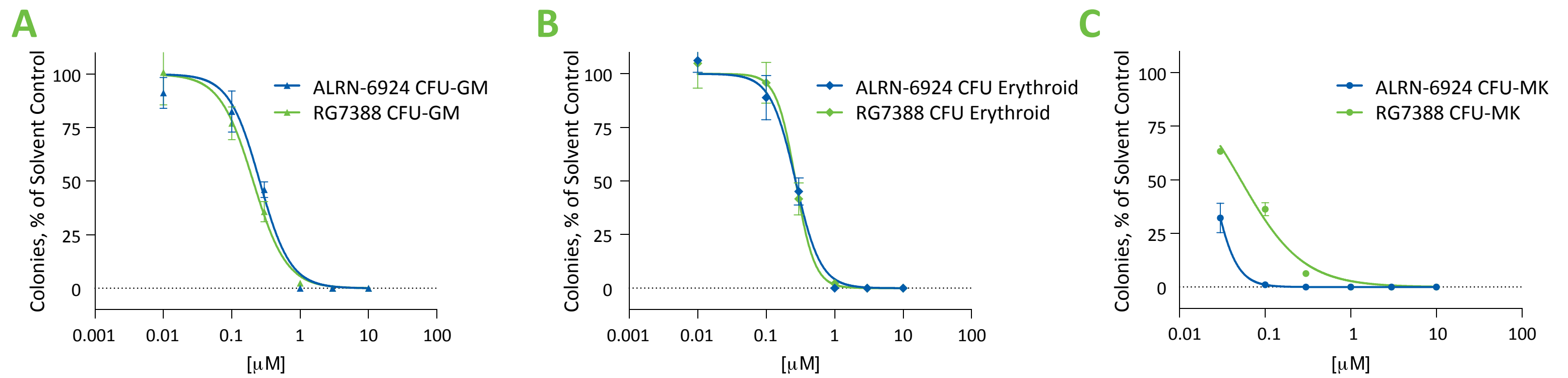


Figure 3: Selective MDM2 Inhibition Potently Disrupts DNA Replication and Induces Apoptosis During Megakaryocyte Development, Abrogates Platelet Production ex vivo



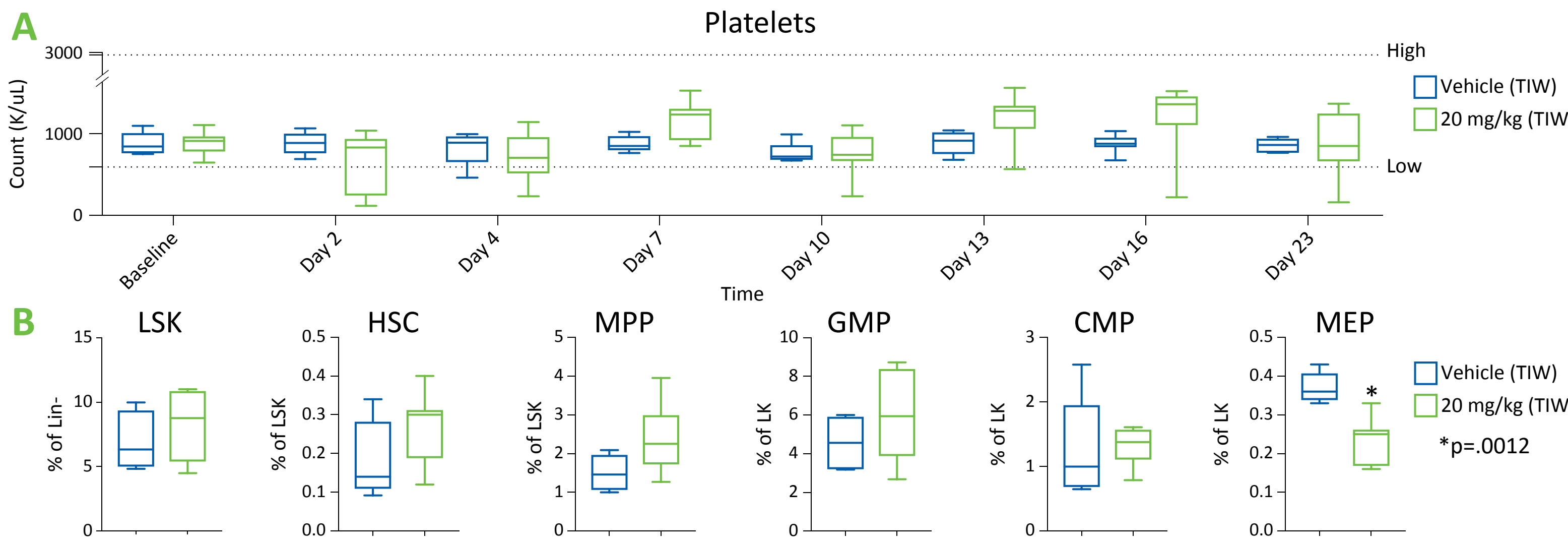
A) Ex vivo primary cell based assay mimicking megakaryocyte development using flow cytometry¹³. Human bone marrow CD34+ cells were cultured in the presence of Tpo and SCF over a 14 day period. **B)** Megakaryocyte development was assessed at the indicated timepoints by measuring CD41 expression. Distinct cell populations were defined as CD41dim and low side scatter (early megakaryocyte), CD41mid and low side scatter (intermediate megakaryocytes) and CD41bright and high side scatter (mature megakaryocytes). **C)** Western blot assessing the expression of CD41 and CD34 at different times during megakaryocyte development in liquid culture.

Figure 4: ALRN-6924 Potently Disrupts Proliferation of Immature Megakaryocyte Progenitors (CFU-MK) Compared to MDM2 Selective Inhibitor but Demonstrates Equipotent Activity Against Erythroid (CFU-E) and Granulocyte-Monocyte (CFU-GM) Progenitors



Assessment of the effect of ALRN-6924 and RG7388 on human progenitor proliferation and differentiation using a colony forming cell assay. To assess the effects of test compounds on human erythroid and myeloid progenitors, healthy donor bone marrow CD34+ (1.2x10⁶) cells were incubated in methyl cellulose-base semi-solid medium containing ALRN-6924 or RG7388, rhIL3 (10ng/mL) rhGM-CSF (10ng/mL), SCF (50 ng/mL and Epo (3 U/mL) **A)**. CFU-GM or **B)**. CFU-E were enumerated in situ microscopically after 16 days of incubation at 37°C, 5% CO₂. **C)** Assessment of megakaryocyte progenitors in semi-solid collagen-based matrix containing rhIL-3 (10ng/mL), rhIL6 (10ng/mL) and rhTpo (50 ng/mL) and either ALRN-6924 or RG7388 for 16 days. All plots shown normalize response to solvent control (n=3).

Figure 5: ALRN-6924 Reduces the Frequency of Megakaryocyte and Erythroid Progenitors (MEP) but Does Not Induce Thrombocytopenia in C57BL/6 Mice



C57BL6 mice (10 per group) were treated with either vehicle or ALRN-6924 (20 mg/kg 3 times per week (TIW)) for 4 weeks. **A)** Platelet counts assessed by Hemavet analysis of peripheral blood obtain from serial bleedings (submandibular vein puncture) on the specified days throughout the duration of treatment. **B)** FACS analysis of mouse hematopoietic stem and progenitors frequencies after treatment with ALRN-6924 (20mg/kg) TIW for 4 weeks (LSK: Lin- Sca1+ Kit+, Hematopoietic Stem Cells (HSC: LSK CD150+ CD48-), Multipotent Progenitors (MPP: LSK CD150- CD48+), Granulocyte-monocyte Progenitors (GMP: CD34^{high} CD16/32^{high}) Common Myeloid Progenitors (CMP: CD34^{mid} CD16/32^{mid}) Megakaryocyte-erythroid Progenitors (MEP: CD34^{low} CD16/32^{low}).

Conclusions

- ALRN-6924 potently inhibits the clonogenic capacity of megakaryocyte progenitors in MK-CFU assays but it is less potent during the process of megakaryocyte maturation than the MDM2-only inhibitor RG7388. Similarly, mice treated with ALRN-6924 showed a statistically significant reduction in MEP frequency but did not develop thrombocytopenia.
- These findings suggest that the disruption of later stages of megakaryocyte maturation with an MDM2-selective inhibitor more severely affects platelet production, ultimately resulting in thrombocytopenia. However, inhibition of less mature megakaryocyte progenitors, prior to entering terminal maturation, appears to be better tolerated. One explanation may be that ALRN-6924 triggers compensatory mechanisms resulting in increased output during terminal maturation of megakaryocytes or alternative stem/progenitor differentiation pathways bypassing phenotypic MEPs.
- These results may explain the differences in TCP grades and frequencies from published solid tumor trials for ALRN-6924 vs. MDM2-only inhibitors, and suggests that MDMX in bone marrow cells, where MDMX RNA expression is the highest of all 37 normal tissues reported in the Protein Atlas¹⁴, may contribute to these differential effects on bone marrow toxicity. Furthermore, these studies suggest functional differences between MDMX and MDM2 during megakaryopoiesis requiring further characterization.
- In collaboration with Pfizer, ALRN-6924, is currently being tested in combination with palbociclib in MDM2-amplified cancers (NCT02264613). And another Phase 1b/2 clinical trial is being planned to evaluate ALRN-6924 as a myelopreservative agent to protect against chemotherapy-induced toxicity.

References

- Meric-Berstam F, et al. Phase I trial of a novel stapled peptide ALRN-6924 disrupting MDMX and MDM2-mediated inhibition of WT p53 in patients with solid tumors and lymphomas. *J. Clin. Oncol.* 35 (15):2505-2505 (2017).
- Meric-Berstam F, et al. ASCO 2017 presentation: http://phare.aileronrx.com/presentations/ALRN-6924_Final_Slides_ASCO_02jun2017.pdf
- Shutov, A. R., et al. Preliminary results of the stapled peptide ALRN-6924, a dual inhibitor of MDMX and MDM2, in two phase I dose expansion cohorts in relapsed/refractory TP53 wild-type peripheral T-cell lymphoma. *Blood* 125(Suppl 1):3623 (2018).
- Briegleb, K. et al. Unravelling mechanisms of p53-mediated tumour suppression. *Nat. Rev. Cancer* 14 (5):359-70 (2014).
- A phase I study of the MDM2 inhibitor AMG 232 in patients with advanced p53 wild-type (p53WT) solid tumors or multiple myeloma. Abstract S34. AACR NC EORTC (2016).
- A phase I dose escalation study of the MDM2 inhibitor DS-3032b in patients with advanced solid tumors and lymphomas. full poster, Abstract 827. AACR NC EORTC (2016).
- A phase I study of MDM2 inhibitor Midaglitinib (DS-3032b) in patients with well-differentiated liposarcoma, solid tumors, and lymphomas. Abstract 15114. ASCO (2018).
- Dose and regimen feeding Phase I Study of NVP-HDM201 in Patients With TP53 Wild-type Advanced Tumors. full poster, Abstract 991. EHA (2018).
- Phase I study of NVP-HDM201 in patients with TP53 wild-type advanced solid tumors. full poster, CT150, oral presentation, AACR (2017).
- A phase I, open-label, multicenter, dose-escalation phase I study of the MDM2 antagonist RO689921, a pegylated prodrug of idasanutlin, for intravenous (IV) administration in patients with advanced solid tumors. Abstract 9016. AACR NC EORTC (2016).
- Phase I dose escalation, food-effect and biomarker study of RG7388, a more potent second-generation MDM2 antagonist in patients (pts) with solid tumors. Abstract 2535. ASCO (2014).
- A phase I trial of the human double minute 2 (MDM2) inhibitor MK-8242 in patients (pts) with advanced solid tumors. Abstract 10562. ASCO (2015).
- An ex vivo megakaryocyte platform to evaluate the effects of compounds on different stages of platelet development. dos Santos S et al. Society of Toxicology 54th Annual Meeting & ToxExpo (2015). Poster Abstract # 402. <https://www.astm.org/abstracts/ENSG000000186625-MDM2a.html>

