

The combination of statins and dipyridamole is effective preclinically in multiple myeloma, acute myelogenous leukemia and breast cancer

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Abstract

Statins have been utilized for years to treat hyperlipidemia by inhibiting the rate-limiting enzyme of the mevalonate (MVA) pathway, 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR). Preclinical evidence demonstrates statins possess anti-cancer properties against a wide range of tumours but not normal cells. We aim to identify compounds which potentiate the anti-cancer effects of statins. This will uncover novel molecular pathways and/or targets that can be exploited in combination with the MVA pathway to maximize tumour cell death. Towards this aim, a pilot 100 compound library, composed of off-patent pharmacologically active drugs clinically used for a wide spectrum of diseases was screened in the multiple myeloma (MM) KMS11 cell line. Dipyridamole (DP), a commonly prescribed anti-platelet agent, potentiated the anti-cancer effects of atorvastatin. The DP-statin combination was synergistic and capable of inducing apoptosis in a variety of acute myelogenous leukemia (AML), MM and breast cancer cell lines. The DP-statin combination also induced apoptosis in primary AML patient samples, but was not toxic to normal peripheral blood stem cells (PBSCs). In an *in vivo* AML tumour model, the DP-statin combination was found to be effective at inhibiting tumour growth.

DP is known to elicit numerous effects, including, phosphodiesterase (PDE) inhibition. In AML cell lines, activators of the protein kinase A (PKA) pathway, also induced apoptosis in combination with statins similar to DP. Interestingly, the DP-statin combination prevented the increase of HMGCR, which occurs following statin treatment as part of a classic feedback response. Further mechanistic investigations to determine how DP potentiates statin-induced apoptosis are underway. As both statins and DP are pre-approved for use in humans, off-patent, and readily available, they have the potential to directly impact patient care.

Statins target the mevalonate pathway

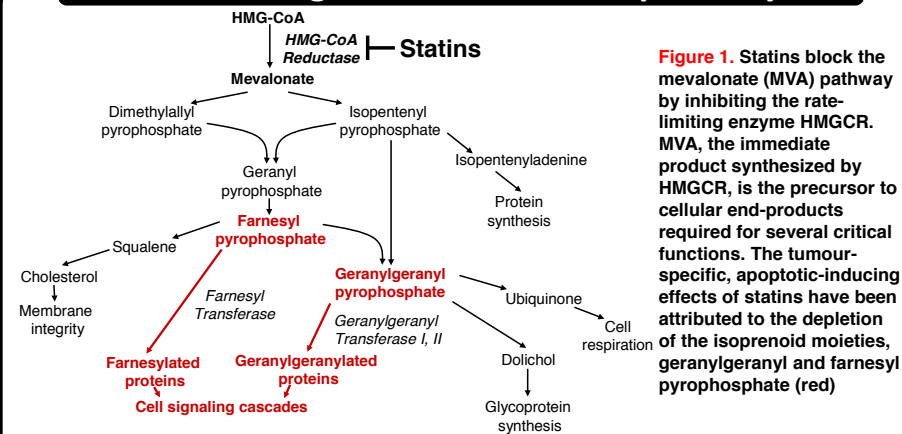
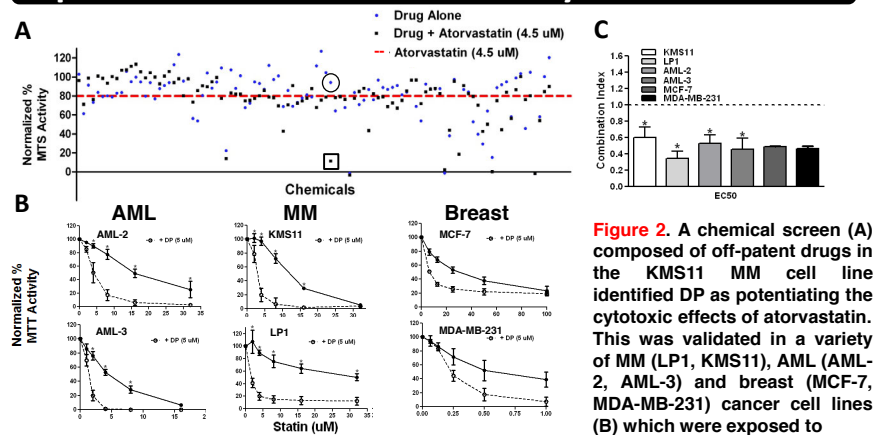


Figure 1. Statins block the mevalonate (MVA) pathway by inhibiting the rate-limiting enzyme HMGCR. MVA, the immediate product synthesized by HMGCR, is the precursor to cellular end-products required for several critical functions. The tumour-specific, apoptotic-inducing effects of statins have been attributed to the depletion of the isoprenoid moieties, geranylgeranyl and farnesyl pyrophosphate (red).

Dipyridamole was identified in a screen as a potentiator of statin-induced cytotoxic effects



the indicated compounds for 48 (AML and MM) or 72 (breast) hrs with cytotoxicity assessed by MTT assay. The DP-statin combination was synergistic in the MM, AML and breast cancer cell lines (C). Drug treatments were performed individually and in a fixed ratio. Synergy was assessed using Chou and Talalay's combination index. $n = 2-4$ * $p < 0.05$, student's t-test, error bars indicate standard deviation (SD).

The dipyridamole-statin combination induces apoptosis in cancer cells and AML patient samples

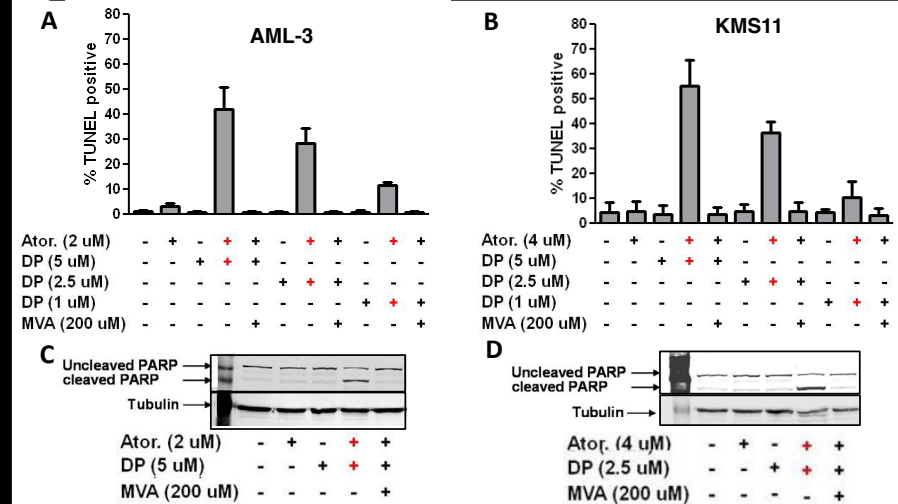


Figure 3. DP and atorvastatin induced apoptosis in the AML-3 and KMS11 cell lines as determined by TUNEL (A,B) and PARP cleavage (C,D) respectively in an MVA-dependent manner. Assays were performed following 48 hrs of exposure at the indicated concentrations $n = 3-4$, error bars indicate SD.

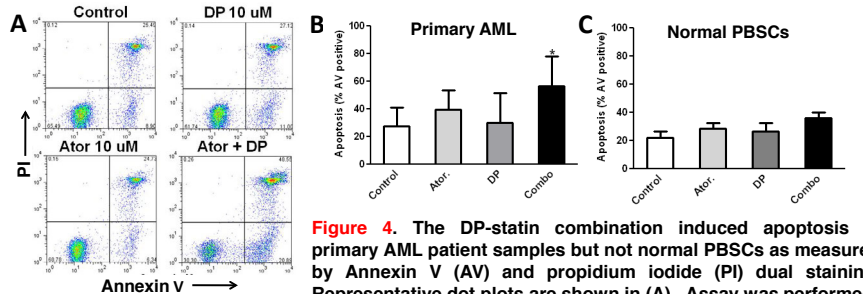


Figure 4. The DP-statin combination induced apoptosis in primary AML patient samples but not normal PBSCs as measured by Annexin V (AV) and propidium iodide (PI) dual staining. Representative dot plots are shown in (A). Assay was performed following 48 hrs of compound exposure: Ator. (atorvastatin 5 μ M for AML and 10 μ M for PBSCs), DP (10 μ M). Apoptosis was determined by summing AV+/PI- (early apoptotic) and AV+/PI+ (late apoptotic) events. $n = 5$ AML patient (B) and $n = 4$ normal PBSCs (C) donors, * $p < 0.05$, 1 way ANOVA with using Tukey's post-hoc test, error bars indicating SD.

The statin-dipyridamole combination reduces tumour growth *in vivo*

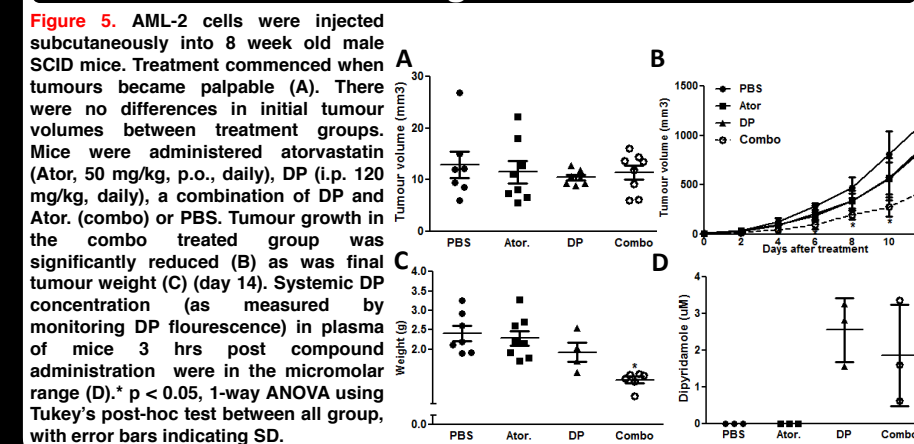


Figure 5. AML-2 cells were injected subcutaneously into 8 week old male SCID mice. Treatment commenced when tumours became palpable (A). There were no differences in initial tumour volumes between treatment groups. Mice were administered atorvastatin (Ator, 50 mg/kg, p.o., daily), DP (i.p. 120 mg/kg, daily), a combination of DP and Ator. (combo) or PBS. Tumour growth in the combo treated group was significantly reduced (B) as was final tumour weight (C) (day 14). Systemic DP concentration (as measured by monitoring DP fluorescence) in plasma of mice 3 hrs post compound administration were in the micromolar range (D). * $p < 0.05$, 1-way ANOVA using Tukey's post-hoc test between all group, with error bars indicating SD.

Pharmacological modulation of the PKA pathway potentiates statin-induced anti-tumour effects

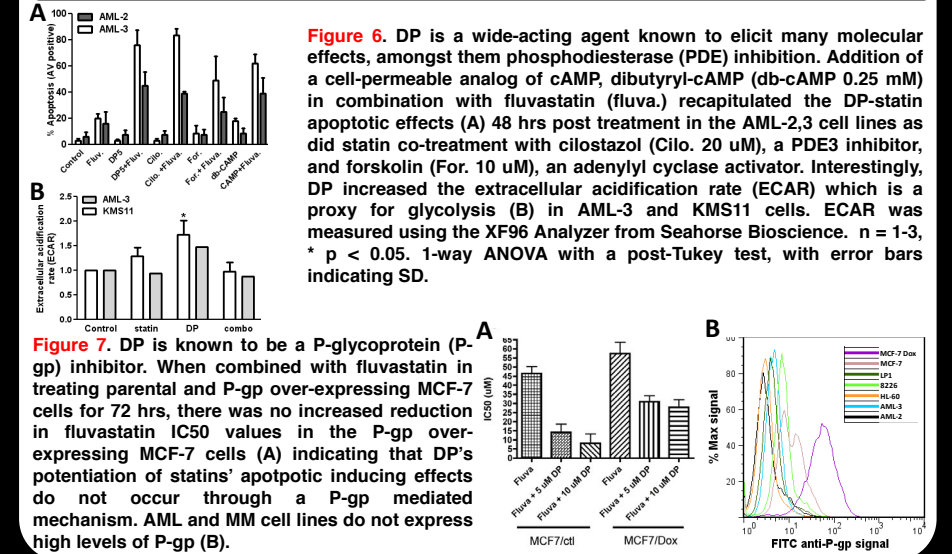


Figure 6. DP is a wide-acting agent known to elicit many molecular effects, amongst them phosphodiesterase (PDE) inhibition. Addition of a cell-permeable analog of cAMP, dibutyryl-cAMP (db-cAMP 0.25 mM) in combination with fluvastatin (fluva.) recapitulated the DP-statin apoptotic effects (A) 48 hrs post treatment in the AML-2,3 cell lines as did statin co-treatment with cilostazol (Cilo. 20 μ M), a PDE3 inhibitor, and forskolin (For. 10 μ M), an adenylyl cyclase activator. Interestingly, DP increased the extracellular acidification rate (ECAR) which is a proxy for glycolysis (B) in AML-3 and KMS11 cells. ECAR was measured using the XF96 Analyzer from Seahorse Bioscience. $n = 1-3$, * $p < 0.05$. 1-way ANOVA with a post-Tukey test, with error bars indicating SD.

The dipyridamole-statin combination affects the sterol-feedback loop

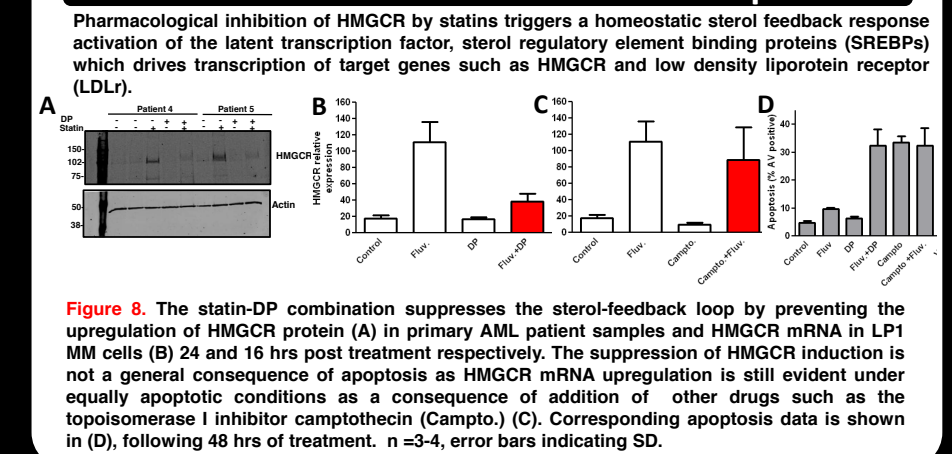


Figure 8. The statin-DP combination suppresses the sterol-feedback loop by preventing the upregulation of HMGR protein (A) in primary AML patient samples and HMGR mRNA in LP1 MM cells (B) 24 and 16 hrs post treatment respectively. The suppression of HMGR induction is not a general consequence of apoptosis as HMGR mRNA upregulation is still evident under equally apoptotic conditions as a consequence of other drugs such as the topoisomerase I inhibitor camptothecin (Campto.) (C). Corresponding apoptosis data is shown in (D), following 48 hrs of treatment. $n = 3-4$, error bars indicating SD.

Summary

- A chemical screen identified dipyridamole (DP), a commonly prescribed anti-platelet agent as potentiating the apoptosis inducing effects of statins.
- Characterization in AML and MM cell lines as well as primary AML patient samples demonstrated the statin-DP combination to be synergistic and capable of potentiating statin-induced apoptosis.
- The combination was efficacious in abrogating tumour growth *in vivo*.
- In AML cell lines, activation of the PKA pathway using pharmacological inhibitors/activators, potentiated the apoptotic-inducing effects of statins.
- The DP-statin combination suppressed the upregulation of HMGR in primary AML patient samples and cell lines providing a potential explanation for the combination's apoptotic inducing effects at a molecular level.
- As both DP and statins are FDA-approved, safe, affordable and readily available, their translation into cancer patient care has the potential to be rapid.

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