

INTRODUCTION

- Purine nucleoside phosphorylase (PNP)-deficient children exhibit profound impairment in T-cell immunity. B-cell dysfunction noted in few patients (30%); this rare clinical condition provides a model for the development of specific inhibitors of PNP in autoimmune disease and hematological malignancies.^{1,2}
- Mechanism: deoxyguanosine (dGuo), produced by purine metabolism, is rapidly degraded by PNP into guanine and deoxyribose sugar. Inhibition of PNP leads to elevation of plasma dGuo, which is then metabolized by deoxycytidine kinase eventually into deoxyguanosine triphosphate (dGTP). dGTP accumulates intracellularly, resulting in an imbalance in intracellular deoxynucleotide pools, causing apoptosis (Figure 1).
- Although PNP is present in all organs, the PNP inhibition mainly affects lymphocytes due to inherently greater phosphorylation of dGuo and slower catabolism of dGTP in lymphocytes.³
- Forodesine (BCX-1777) is a first generation potent transition state inhibitor of PNP that is currently in phase II clinical trials for Cutaneous T-cell lymphoma.⁴
- BCX-4208 (RO5092888; Figure 2) is a second generation potent transition-state inhibitor of PNP.⁵ The purpose of the present study is to investigate the following:
 - Effect of BCX-4208 on various cell subsets and proliferation of human lymphocytes
 - Understand the relationship between dGTP and efficacy
 - Pharmacokinetic and pharmacodynamic effects in mice

Figure 1. Mechanism of action:

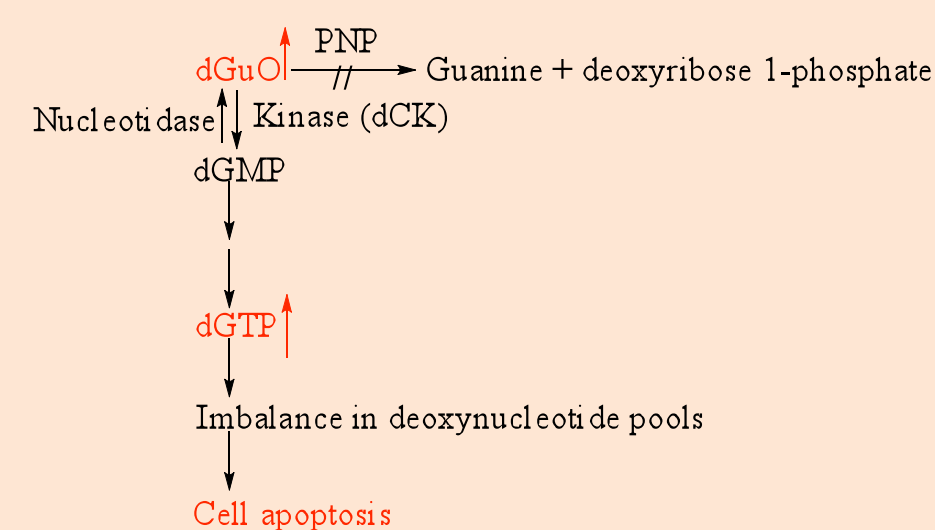
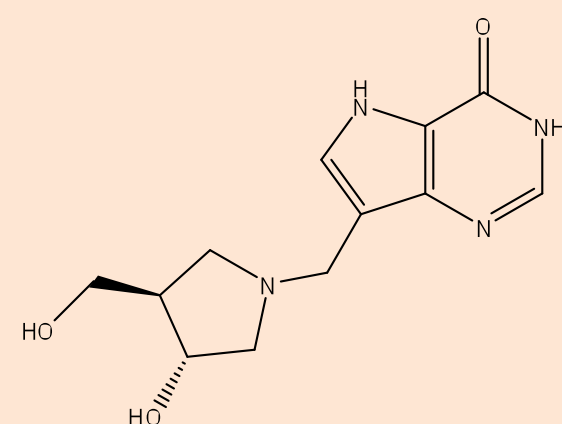


Figure 2. Structure of BCX-4208 (RO5092888)



MATERIALS AND METHODS

PNP assay and lymphoproliferative studies were conducted as described.⁶ dGTP levels in lymphocytes were measured using a polymerase assay. Cell subset analysis was performed by flow cytometry using Guava EasyCyte. In vivo pharmacologic activity was conducted as follows: Groups of four female mice received a single oral dose of BCX-4208 in saline and at various times blood was drawn, centrifuged and plasma was analyzed for BCX-4208 and dGuo using reverse phase HPLC.

RESULTS

Table 1. BCX-4208 is a potent inhibitor of PNP from various species

Species	IC ₅₀ (nM)
Human	0.52 ± 0.08
Rat	1.05 ± 0.03
Mouse	1.75 ± 0.015
Monkey (cynomolgous)	0.65 ± 0.22

*Values are mean ± SEM of 2 independent experiments (human PNP, n=4)

Table 2: BCX-4208 in the presence of dGuo inhibits proliferation of human lymphocytes activated by various agents. Neither BCX-4208 alone nor dGuo alone inhibits proliferation of lymphocytes.

Activating agent	IC ₅₀ (μM)*	
	BCX-4208 ^a	dGuo ^b
Interleukin-2	0.263 ± 0.04 (n=23)	3.12 ± 0.39 (n=22)
Con A	0.73 ± 0.14 (n=10)	ND
Mixed Lymphocyte Reaction (MLR)	0.159 ± 0.03 (n=15)	1.55 ± 0.26 (n=11)

*Values are mean ± SEM; ^ain the presence of 10 μM dGuo; ^bin the presence of 1 μM BCX-4208
ND – not determined

Figure 3. BCX-4208 (3 μM) in the presence of dGuo (10 μM) induces cellular apoptosis not only in T cells (CD3+, CD4+, CD8+), but also in B (CD20+; CD19+) and NK (CD56+) cells

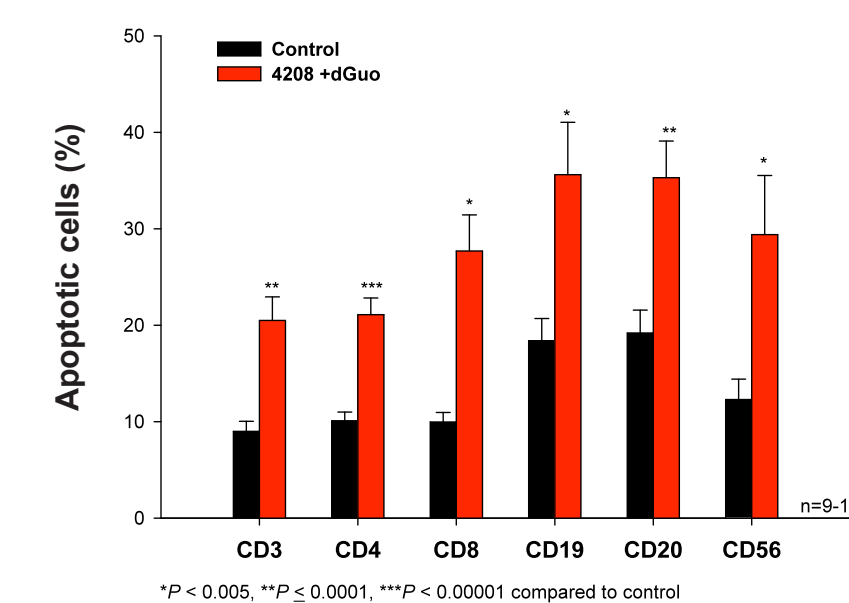


Figure 4. Intracellular increase in dGTP levels after BCX-4208 and dGuo treatment correlates with inhibition of proliferation of lymphocytes.

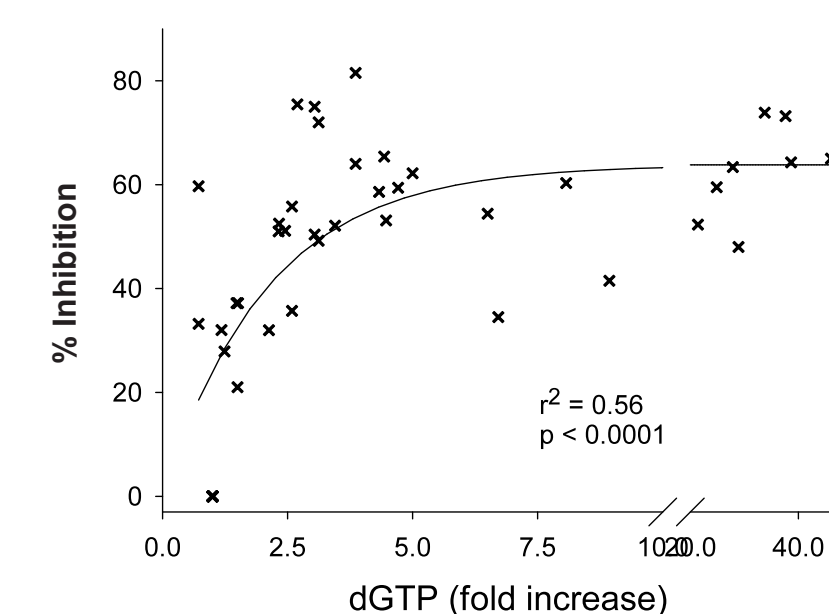
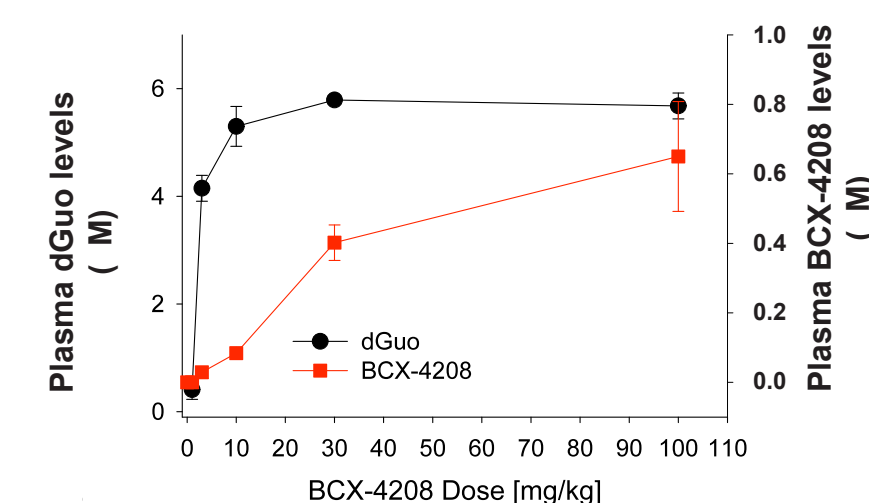


Figure 5. Pharmacokinetics and Pharmacodynamics in Mice. Plasma dGuo levels achieved are similar both to levels seen in PNP deficient patients and to levels needed to cause inhibition of proliferation of lymphocytes. Beyond the 10 mg/kg dose, no further increase in plasma dGuo was observed suggesting near complete inhibition of PNP enzyme was achieved at 10 mg/kg dose.



SUMMARY

- Potent transition state analog inhibitor of PNP (Table 1)
- Inhibits lymphocyte cell proliferation in vitro (Table 2)
- Induces apoptosis in T, B, and NK cells (Figure 3)
- Inhibition of proliferation is dGTP mediated (Figure 4)
- Orally absorbed and elevates plasma dGuo (Figure 5)

CONCLUSION

Preclinical studies support the evaluation of BCX-4208 in the treatment of T-cell and B-cell mediated autoimmune diseases and hematologic malignancies. BCX-4208 is currently undergoing early clinical investigation in patients with psoriasis (see abstract # 15251).

REFERENCES

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