Abstract # 2820

Effects of a CDK 4/6 inhibitor, G1T38, in androgen receptor sensitive and resistant models of castrate resistant prostate cancer

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Abstract

Introduction: Resistance to endocrine therapies, via expression of mutations or variants of the androgen receptor (AR), remains an impediment to enduring therapeutic responses in advanced castration resistant prostate cancer (CRPC). AR-dependent upregulation of cyclins leads to activation of the Cyclin D1-cyclin dependent kinase 4/6 (CDK 4/6) complex and cell proliferation, suggesting that targeting of this axis may be effective in CRPC. We have developed a series of potent and selective CDK 4/6 inhibitors, of which G1T38 has an IC50 in the low nanomolar range, and >3000 fold selectivity for CDK4/6 over CDK 2/cyclin A/E complexes. The efficacy of G1T38 was evaluated using models of CRPC, the results of which have significant clinical implications.

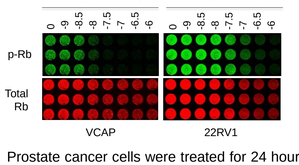
Methods: For proliferation assays, cells were treated for 5-10 days with G1T38 or standard of care comparators prior to quantitation of cell number/viability. Cell cycle progression and apoptosis were assayed by flow cytometry using propidium iodide or Annexin V/Sytox red staining, respectively. Orchiectomized Nu/Nu or NOD SCID Gamma mice bearing 22RV1 or LnCAP-AR-F876L xenograft tumors, respectively, were treated with G1T38 or clinically relevant comparators.

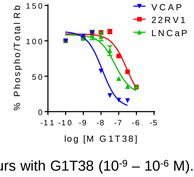
Results: G1T38 inhibited the growth of prostate cancer cell lines expressing wild type AR (LnCAP and VCAP), resistance associated AR variant AR v7 (22RV1 and LnCAP95), and LnCAP cells overexpressing the Enzalutamide resistant AR mutation F876L. Corresponding decreases in cell cycle G0/G1 progression and in Rb phosphorylation (S807/811) were observed in all cell lines tested, whereas no changes were observed in Cyclin D1, E or Cdk2, 4, or 6 expression. Growth inhibition by G1T38 was dependent on Rb, and not AR, status as DU145 (AR-/Rb-) cells were not growth inhibited by G1T38, while PC3 (AR-/Rb+) were growth arrested. Treatment of G1T38 in combination with the anti-androgen Enzalutamide increased the sensitivity of VCAP and 22RV1 cells to growth inhibition. Interestingly, Enzalutamide in combination with G1T38 at high doses produced a synergistic apoptotic effect in LnCAP, VCAP, and 22RV1 cells, which could be attenuated by the caspase inhibitor Q-VD-OPH. The growth of 22RV1 cells and LnCAP AR F876L cells were assessed when propagated as xenografts. In 22RV1 cells, pharmacologic CDK 4/6 inhibition, significantly resulted in tumor regression compared to vehicle or docetaxel. In the LnCAP-AR-F876L xenograft model, tumor growth and doubling time was significantly decreased by low and high dose G1T38 treatment compared to control and Enzalutamide.

Conclusions: The CDK 4/6 inhibitor G1T38 exerts anti-proliferative effects in relevant models of CRPC when used as a stand-alone agent or when tested in combination with Enzalutamide. G1T38 inhibited xenograft tumor growth to a greater extent than other available therapies, highlighting the utility of CDK 4/6 inhibition in prostate cancer and a potential new paradigm in CRPC treatment.

Results

1 G1T38 inhibits CDK 4/6 activity in androgen sensitive and insensitive models of castration resistant prostate cancer.



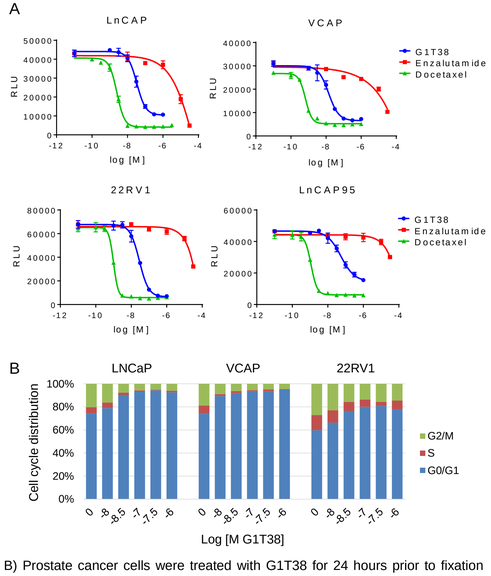


Prostate cancer cells were treated for 24 hours with G1T38 (10^{-9} – 10^{-6} M). Total Rb and pRb protein levels were detected in fixed cells by in-cell western.

Results

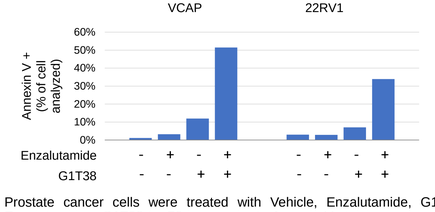
2 G1T38 inhibits the proliferation of prostate cancer cells by inducing cell cycle arrest.

A) Prostate cancer cells were treated with G1T38, docetaxel or Enzalutamide for 5-7 days. DNA content was used as a measure of cell proliferation.



B) Prostate cancer cells were treated with G1T38 for 24 hours prior to fixation and propidium iodide staining, followed by analysis using flow cytometry.

3 Combination of G1T38 with Enzalutamide induces apoptosis in prostate cancer cell models.

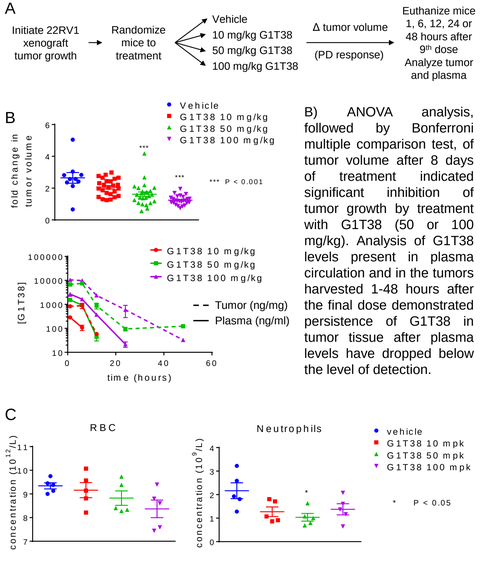


Prostate cancer cells were treated with Vehicle, Enzalutamide, G1T38 or Enzalutamide + G1T38 for 72 hours prior to Annexin V staining and analysis by flow cytometry.

Results

4 G1T38 exhibits favorable PK and PD in 22RV1 xenograft tumors with only mild myelosuppression.

A) Castrated male nu/nu mice bearing 22RV1 xenograft tumors were randomized to treatment with vehicle, G1T38 (10, 50 or 100 mg/kg) p.o. daily. Mice were euthanized 1, 6, 12, 24 or 48 hours after the 9th dose, and plasma and tumor drug levels of G1T38 were analyzed by LC-MS/MS.



B) ANOVA analysis, followed by Bonferroni multiple comparison test, of tumor volume after 8 days of treatment indicated significant inhibition of tumor growth by treatment with G1T38 (50 or 100 mg/kg). Analysis of G1T38 levels present in plasma circulation and in the tumors harvested 1-48 hours after the final dose demonstrated persistence of G1T38 in tumor tissue after plasma levels have dropped below the level of detection.

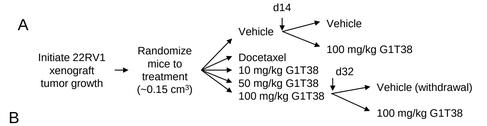
C) G1T38 mildly repressed neutrophil numbers while having no effect on red blood cells (RBCs) as indicated by CBC analysis of whole blood retained from animals euthanized 1 hour after treatment in the above PK/PD analysis (ANOVA analysis and Bonferroni multiple comparison test).

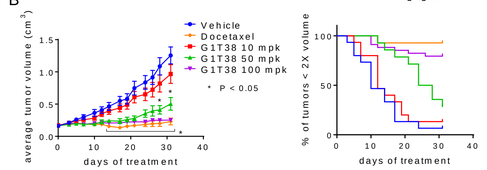
D) Bone marrow isolated from animals euthanized 24 or 48 hours after the final treatment in the above PK/PD analysis was analyzed by immunostaining (CD11b and Ly6G+) and flow cytometry. G1T38 repressed neutrophil number (ANOVA analysis and Bonferroni multiple comparison test).

Results

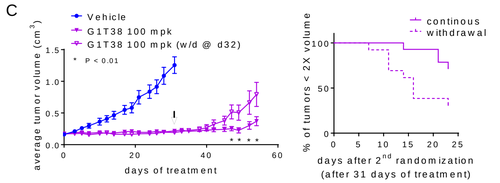
5 G1T38 inhibits the growth of 22RV1 xenograft tumors in a dose dependent manner with good tolerability.

A) Castrated male nu/nu mice bearing 22RV1 xenograft tumors were randomized to treatment with vehicle, docetaxel (20 mg/kg i.p. weekly), or G1T38 (10, 50 or 100 mg/kg) p.o. daily. One cohort of mice was randomized to treatment with 100 mg/kg G1T38 after 14d of treatment with vehicle. After 31 days of treatment, mice receiving 100 mg/kg G1T38 treatment since day 0 were re-randomized to treatment with vehicle (withdrawal) or continued treatment with G1T38. **Notably, no significant changes in the body weight of animals receiving G1T38 were observed** (not shown).

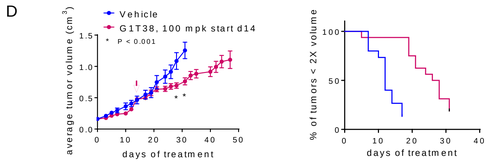




B) Both time to progression analysis and 2-way ANOVA comparison of average tumor volumes throughout treatment, followed by Bonferroni multiple comparison test, indicated significant tumor growth inhibition by 50 and 100 mg/kg G1T38, and by the docetaxel control.



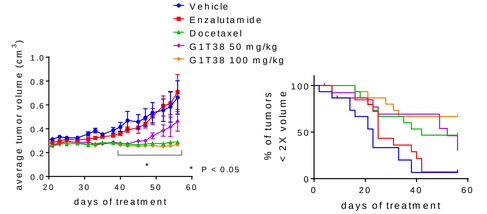
C) 2-way ANOVA and time to progression analysis indicate relapse and renewed tumor growth in mice withdrawn from treatment after 31 days of receiving 100 mg/kg G1T38.



D) 2-way ANOVA and time to progression analysis indicate incomplete inhibition of tumor growth in animals re-randomized to 100 mg/kg G1T38 at d14.

Results

6 G1T38 inhibits the growth of LNCaP tumors expressing the Enzalutamide associated AR-F876L mutation.



Castrated male NSG mice bearing LNCaP-AR-F876L xenograft tumors were randomized to treatment with vehicle, Enzalutamide (30 mg/kg p.o. daily), G1T38 (50, or 100 mg/kg p.o. daily), or docetaxel (20 mg/kg i.p. weekly. Both time to progression analysis and 2-way ANOVA comparison of average tumor volumes throughout treatment, followed by Bonferroni multiple comparison test, indicated significant tumor growth inhibition by 50 and 100 mg/kg G1T38, and by the docetaxel control.

Summary

- CDK 4/6 inhibitor G1T38 inhibits the growth of several models of castration resistant prostate cancer in vitro through on target efficacy that correlates with the IC_{50} for target inhibition.
- Combination of G1T38 with Enzalutamide was found to induce apoptosis in prostate cancer cells in vitro.
- G1T38 exhibits a favorable PK profile in that the compound accumulates in tumor, but not in plasma.
- G1T38 was found to inhibit the growth of clinically relevant models of advanced castrate resistant prostate cancer, including:
  - 22RV1 xenograft tumors (AR-V7 +)
  - LNCaP-AR-F876L xenograft tumors (expressing the F876L androgen receptor variant clinically associated with resistance)
- G1T38 will enter Phase 1 Healthy volunteer trials in the second quarter of 2016.

Acknowledgements

- Funding was provided by G1 Therapeutics, Inc., via a sponsored research agreement.
- Preclinical Characterization of G1T38 is presented in Poster #2824