Comparing Everolimus to Cetuximab as Adjuvant Therapy: TP53 Mutant HNSCC

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Abstract

Introduction - Patients with TP53 mutant head and neck squamous cell carcinoma (p53mHNSCC) have poor response to treatment due to resistance to chemoradiotherapy. Platinum therapies have a high toxicity profile and the alternative, cetuximab, has a high resistance profile. Everolimus (an mTOR inhibitor) has had promising results in Phase II trials. Therefore, we compare the efficacy of cetuximab to everolimus in inhibiting growth of p53mHNSCC in a minimal residual disease (MRD) tumor xenograft model.

Methods - In this in vivo nude mouse trial conducted from Aug 2021 to Oct 2021, athymic nude mice were implanted with p53mHNSCC FaDu cells and randomized into vehicle, cetuximab, or everolimus treatment groups. The vehicle group received saline (n=5), the cetuximab group (n=5) received biweekly cetuximab intraperitoneally (0.25 mg) and the everolimus group (n=5) received 5 mg/kg/b.w. daily via oral gavage. Treatment was started three days after tumor implantation to mimic MRD models. The tumor volumes were measured twice weekly for 90 days or until humane endpoints were met. Tumor growth charts were made and statistical analyses run.

Results - Our results demonstrated equal tumor inhibition by everolimus and Cetuximab on the growth of FaDu tumor cell xenografts for 40 days while all vehicle group mice were sacrificed in this time period due to meeting endpoint criteria. However, by day 55 the average tumor volume for the everolimus treatment group was 167 mm^3 (CI95% 156-177) while the average volume of the cetuximab treatment group was 388 mm^3 (CI95% 370-405) during the same time ($p < 0.001$). FaDu xenografts treated with Cetuximab developed resistant tumors while the xenografts treated with everolimus failed to elicit any resistance to treatment and exhibited sustained sensitivity for 13 weeks. Taken together, this study establishes a lack of treatment resistance to medium term everolimus treatment in p53mHNSCC when compared to cetuximab.

Conclusions - Everolimus holds promise as an effective adjuvant therapy for p53mHNSCC and tumor resistance did not develop when compared to cetuximab.

Introduction

Head and Neck Squamous Cell Carcinoma patients have a dismal 5-year survival rate with up to 60% of patients presenting with locally advanced disease. Most aggressive tumors have TP53 mutations appearing in 75-85% of cases. Currently, limited effective treatment options exist for TP53 mutant head and neck squamous cell carcinoma (p53mHSCC) and the mainstay of chemotherapy, platinum-based agents, have high toxicity profiles. Additionally, p53mHNSCC exhibits a high level of resistance to the alternative chemotherapeutic, cetuximab. Use of mTOR inhibitors (mTORi) in p53mHNSCC work downstream from EGFR (Figure 1) and a recent phase II multi-institutional clinical trial has determined that the mTORi everolimus is a viable candidate to repress tumor growth in p53mHNSCC.

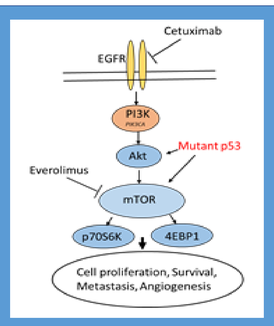
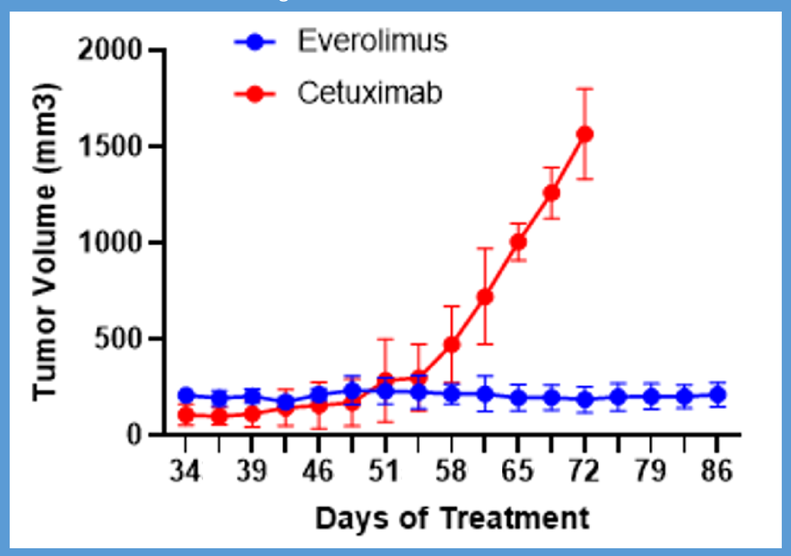


Figure 1: **The PIK3CA/AKT/mTOR pathway.** The PIK3CA/AKT/mTOR pathway is an intracellular signaling pathway responsible for a myriad of cellular activity including cell proliferation, propagation, survival and angiogenesis. It is commonly upregulated in TP53 mutant HNSCC. Cetuximab is an EGFR inhibitor that works upstream to the PIK3CA/AKT/mTOR pathway and is easily overcome by downstream resistance. The mTORi everolimus works on the downstream end of this pathway, as seen in the figure, and has the potential to slow tumor growth and overcome cetuximab resistance in HNSCC.

Figure 2: Growth of TP53 mutant HNSCC tumors treated with Cetuximab and everolimus. Cetuximab resistance begins to develop at day 50 while tumors treated with everolimus do not show growth.



Methods and Materials

A cohort of 15 athymic nude mice were implanted with FaDu p53mHNSCC cells in their bilateral flanks. Mice were then selected to receive either vehicle (n=5), cetuximab (n=5), or everolimus treatment (n=5). The vehicle group received saline only. The mice treated with cetuximab were given biweekly intraperitoneal injections (0.25 mg) while the everolimus treatment branch mice were given 5 mg/kg/b.w daily via oral gavage. To mimic clinical conditions, we utilized a minimal residual disease model, where treatment began within 3 days post-tumor implantation. Research was conducted in compliance with LSUHSC Institutional Animal Care & Use Committee guidelines under the U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals. Tumors were measured biweekly using a caliper and volume was calculated based on long and short axis ellipsoid volume calculation. The project was carried out for 90 days or until humane endpoint criteria were met. Following sacrifice, tumors were harvested and frozen. Tumors were processed and protein lysates analyzed by Western Blotting to analyze the molecular mechanisms of everolimus and the effect on the mTOR pathway (Figure 3) as well as angiogenic growth factor proteins (Figure 4).

Results

All vehicle mice were humanely sacrificed within 40 days due to rapid tumor growth. It was noted at 40 days that the mice receiving cetuximab for tumor suppression began to show signs of tumor progression and by day 55 all tumors treated with cetuximab had shown significant progression. The average tumor volume for the everolimus treatment group was 167 mm^3 (CI95% 156-177) while the average volume of the cetuximab treatment group was 388 mm^3 (CI95% 370-405) during the same time ($p < 0.001$). The mice in the cetuximab treatment group showed tumor progression and at day 72, the decision was made to sacrifice the cetuximab treatment group for humane endpoint purposes. The everolimus treatment group exhibited tumor repression in all observed tumors until experiment end.

Western blotting analysis of tumors showed downregulation of the activated mTOR protein and its corresponding pathway markers. Specifically, angiogenic and lymphangiogenic growth factors HIF-1a, VEGF-A and VEGF-C were downregulated.

Figure 3. Western Blot of mTORi pathway proteins.

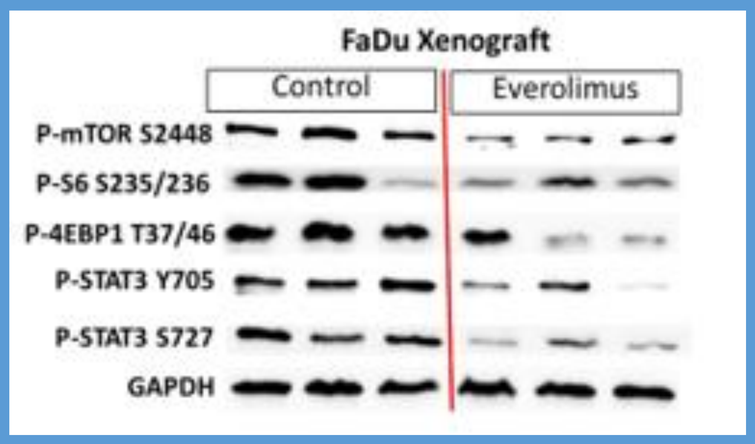
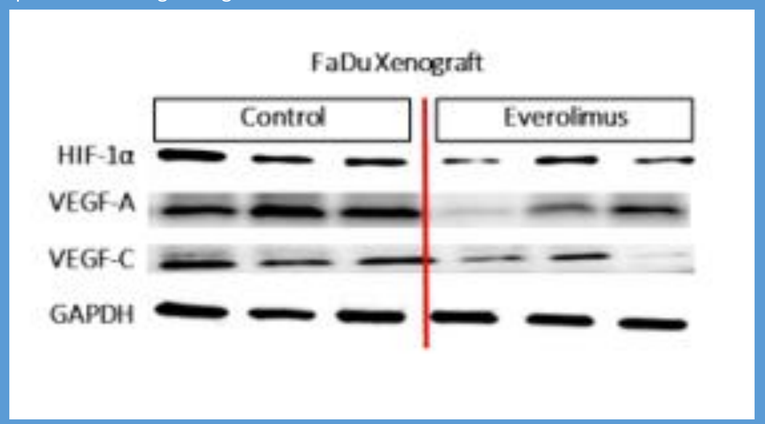


Figure 4: Western blot of angiogenic proteins involved in vascular proliferation in growing tumors.



Discussion

Our results demonstrate everolimus is an effective treatment for p53mHNSCC and maintains activity of tumor suppression in a minimal residual disease model. Cetuximab resistance is a well known phenomenon in p53mHNSCC and the rapid development of treatment resistance was observed in our study. A phase 2 trial in human subjects demonstrated that oral everolimus is a safe treatment in p53mHNSCC patients and can increase 2-year progression free survival. A study by Wang et al. demonstrated everolimus was also effective in xenograft models with PIK3A and RAS mutations.

The mTOR protein has a myriad of effects on cellular growth and the full extent of its pathways are still being elucidated. Our results show everolimus is effective at repressing downstream mTOR pathway proteins and could prevent tumor propagation through decreasing vascular and lymphatic flow in p53mHNSCC tumors.

As p53HNSCC has an aggressive profile and has limited treatment options, everolimus shows potential as a treatment option when alternative therapies are not an option.

Conclusions

The mTORi everolimus is a promising treatment in p53mHNSCC where platinum agents are not an option. Its inhibition leads to a decrease in the angiogenic factors involved in tumor growth. Additional studies are needed to elucidate the role of everolimus in treatment resistant high risk p53mHNSCC.

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References

1. Leemans CR, Braakhuis BJ, Brakenhoff RH. The molecular biology of head and neck cancer. Nature reviews cancer. 2011 Jan;11(1):9-22.
2. Zhou G, Liu Z, Myers JN. TP53 Mutations in Head and Neck Squamous Cell Carcinoma and Their Impact on Disease Progression and Treatment Response. *J Cell Biochem.* 2016;117(12):2682-2692. doi:10.1002/jcb.25592
3. Poeta ML, Manola J, Goldwasser MA, et al. TP53 mutations and survival in squamous-cell carcinoma of the head and neck. *N Engl J Med.* 2007;357(25):2552-2561. doi:10.1056/NEJMoa073770
4. Wheeler DL, Dunn EF, Harari PM. Understanding resistance to EGFR inhibitors-impact on future treatment strategies. *Nat Rev Clin Oncol.* 2010;7(9):493-507. doi:10.1038/nrclinonc.2010.97
5. Zhou G, Wang J, Zhao M, Xie TX, Tanaka N, Sano D, Patel AA, Ward AM, Sandulache VC, Jasser SA, Skinner HD, Fitzgerald AL, Osman AA, Wei Y, Xia X, Songyang Z, Mills GB, Hung MC, Caulin C, Liang J, Myers JN. Gain-of-function mutant p53 promotes cell growth and cancer cell metabolism via inhibition of AMPK activation. Mol Cell. 2014 Jun 19;54(6):960-74. doi: 10.1016/j.molcel.2014.04.024. Epub 2014 May 22. PMID: 24857548; PMCID: PMC4067806.
6. Patel J, Nguyen SA, Ogretmen B, Gutkind JS, Nathan CA, Day T. mTOR inhibitor use in head and neck squamous cell carcinoma: A meta-analysis on survival, tumor response, and toxicity. *Laryngoscope Investig Otolaryngol.* 2020;5(2):243-255. Published 2020 Mar 12. doi:10.1002/lio2.370
7. Wang Z, Martin D, Molinolo AA, et al. mTOR co-targeting in cetuximab resistance in head and neck cancers harboring PIK3CA and RAS mutations. *J Natl Cancer Inst.* 2014;106(9):dju215. Published 2014 Aug 5. doi:10.1093/jnci/dju215