CarceSeq detection of a dose- and time-dependent induction of a cancer driver mutation in the mammary DNA of lorcaserin-treated rats

Jennifer Faske1, Matthew Bryant², Meagan Myers1, Xiaobo He2, Florence McLellen2, Todd Bourcier3 and Barbara Parsons1

1Division of Genetic and Molecular Toxicology, 2Office of Scientific Coordination, National Center for Toxicological Research, Jefferson, AR, 3Division of Pharmacology and Toxicology, Office of Cardiology, Hematology, Endocrinology, and Nephrology, Center for Drug Evaluation and Research, US FDA

Abstract

Lorcaserin, a drug for weight management, is a selective agonist of the serotonin (5-hydroxytryptamine) 2c receptor. Although lorcaserin is a non-genotoxic rat carcinogen, FDA approval was granted in part on dose extrapolations considerations. A post-marketing study, CAMELLIA-TIMA, designed to detect potential cardiovascular effects of lorcaserin therapy detected excess cancer risk in the lorcaserin treatment arm. Consequently, a study of lorcaserin-treated rats was conducted to elucidate the mechanism of lorcaserin-induced carcinogenesis and facilitate detection of other carcinogenic operations through the same mechanism in the future. Another study goal was to characterize CarceSeq utility in detecting the neoplasia-related effects of a non-genotoxic carcinogen. CarceSeq is an error-corrected next-generation sequencing method for quantification of panels of hotspot cancer driver mutations (CDMs) and can detect mutations with mutant fractions (MFs) ≥ 0.7%. Female Sprague Dawley rats were treated by gavage daily with 0, 30, or 100 mg/kg lorcaserin, replicating the tumor bioassay doses but with shorter duration treatments of 12 or 24 weeks. Lorcaserin and N-nitroso-lorcaserin were quantified in dosing solutions, terminal plasma and terminal liver samples using ultra-high-performance liquid chromatography-electrospray tandem mass spectrometry. N-nitroso-lorcaserin was not detected. Mammary DNAs (n = 6/group) were used to synthesize PCR products from genes containing known hotspot CDMs (Apc, Braf, Cdk4, Egr1, Ets1, Kras, Nfe2l2, Pik3ca, Setbp1, Sirt1, and Tp53)[3] and variant MFs were quantified by CarceSeq. Considering MFs in all targets, no significant effects of lorcaserin treatment were observed. However, significant induction of Pik3ca H1047R mutation was observed after 12 and 24 weeks of treatment (ANOVA, F(2,9), with greater numbers of mutants and mutants with higher MFs observed in 24-week samples compared to 12-week samples. Given that Pik3ca H1047R mutation can be detected in normal tissues, is the most prevalent mutation in human breast cancer, and occurs in several other cancers, these results suggest lorcaserin-induced carcinogenesis involves promoting the outgrowth of spontaneous-occurring Pik3ca H1047R mutant clones. The underlying mechanism(s) of promotion are under investigation. This study provides proof-of-principle that CarceSeq can be used to detect the effects of a non-genotoxic carcinogen.

Materials and Methods

L-5174 Herceptin was not detected (meaning <10

Results and Discussion

Figure 2. CarceSeq detected >1,100 somatic mutants with MFs ≥ 0.7%. Aside from Pik3ca, MFs were not significantly different across doses and timepoints. Rodent mutations equivalent to the known human breast cancer hotspot mutation, P102CA H1047R are identified in rectangles.

Figure 3. Time- and dose-dependent increase in Pik3ca H1047R MF were observed after 22 (A) and 24 weeks (B) of lorcaserin treatment. Magnitudes of the CarceSeq MF measurements are consistent with lorcaserin-induced clonal expansion of Pik3ca H1047R mutation. DNA samples from rats treated for 24 weeks were analyzed in two independent CarceSeq experiments. The significant correlation observed for replicate measurements was not due to differences in experimental performance. When a Pik3ca H1047R MF was not detected (meaning <10^-9) in a rat, it was not detected in either replicate.

Conclusion

Chemical and nuclear magnetic resonance analyses confirmed: 1) the identity of the test article, 2) the lorcaserin concentrations of dosing solutions, 3) lorcaserin levels in terminal plasma and liver, and 4) that N-nitroso-lorcaserin was not present in any of the samples analyzed. These results confirm that lorcaserin is a non-genotoxic carcinogen and the rat carcinogenicity bioassay conditions were replicated appropriately.

The CarceSeq analysis demonstrated that lorcaserin induced dose-dependent increases in Pik3ca H1047R mutants in rat mammary DNA, with MFs increasing with duration of treatment. This observation has clear human relevance because the Pik3ca H1047R mutation is the single most prevalent point mutation observed in human breast cancer.

Given that lorcaserin is a non-genotoxic carcinogen and Pik3ca H1047R mutation occurs in normal rat and human mammary tissues, these findings demonstrate that lorcaserin induces mammary carcinogenesis by promoting the proliferation of spontaneously-occurring Pik3ca H1047R mutant clones.

This study provides proof-of-principle that CarceSeq can be used to detect the effects of a non-genotoxic carcinogen.