epoxide, followed by glutathione adduct formation and conjugation to NS5B to yield a replication-deficient enzyme.[95]

In Phase 1 clinical trials, tegobuvir provided modest ($1.6\log_{10}$ and $1.95\log_{10}$ at 40 and 120 mg bid) viral load reductions following 8 days of monotherapy, but concerns over QT prolongation observed at the high dose limited future studies to the lower 40 mg dose.[96] NS5B resistance analysis revealed that 58% of patients in the monotherapy trial harbored a Y448H alone or combined with Y452H that seriously compromised the efficacy of the drug. In a Phase 2b clinical trial, supplementing tegobuvir (40 mg bid for 24 or 48 weeks) with PegIFN/RBV enhanced early virologic response but overall did not improve the SVR rate over SOC alone. Y448H in NS5B was the primary resistant mutant in gt1a/1b patients, reverting to wild-type in half the patients following the 12 week end of treatment.[97] *In vitro* experiments performed with the replicon showed that **64** was not cross-resistant to other classes of DAAs and combinations of tegobuvir with protease inhibitors or complementary NS5B inhibitors were either additive or slightly synergistic in curing cells from HCV replicons.[98] For that reason, the safety, tolerability and efficacy of tegobuvir when combined with a protease inhibitor (GS-9256) with and without PegIFN/RBV were investigated in subsequent trials. After 4 weeks of treatment, the two DAA combinations (with and without SOC followed by SOC alone) provided high early virological response rates (EVRs).[99a] High SVR_{12} rates (92%) were reported with 16 weeks of tegobuvir (20 mg bid) + GS-9256 (150 mg bid) with PegIFN/RBV. However, two severe pancytopenia adverse events were reported in two separate trials in patients administered tegobuvir/DAA/PegIFN/RBV, and all ongoing trials involving such quadruple combinations have been stopped.[99b] The most recent report on tegobuvir trials presented 12 week interim results for an interferon-free, all-oral combination of tegobuvir (30 mg bid) + NS5A inhibitor (GS-5885, 30 or 90 mg per day) + a new once-per-day NS3 PI (GS-9451, 200 mg qd) and RBV in gt1 HCV patients.[100] High SVR_4 rates (96%) were observed for patients who stopped therapy at week 12 and breakthrough occurred in only one subject (gt1a).

8.4 Conclusion

HCV NS5B has provided researchers with fertile ground for drug discovery. An unusual diversity of structurally disparate chemotypes has been uncovered over the years that were successfully optimized to provide development candidates. In addition to the nucleoside active-site inhibitors discussed elsewhere in this book (chapter 12), non-nucleoside inhibitors that bind to four distinct allosteric sites on the enzyme (thumb pockets 1 and 2, palm sites 1 and 2) have been identified. Representative compounds from these families are thought to interfere with important NS5B conformational changes at various stages of the initiation steps that are necessary for productive RNA replication. The antiviral efficacy of inhibitors from each of the four allosteric classes has been validated in the clinic, but the emergence of resistant virus has not provided a