administered qd for 3 days at doses of 1, 3, 10, 30 and 90 mg and the effect on viral load was compared with placebo. Plasma exposure of GS-5885 was close to linear with respect to dose, with C_{max} occurring at 4–6 h after drug administration. Quantifiable concentrations were detectable at 24 h after all doses, exceeding the protein binding-adjusted EC_{90} for the less sensitive G-1a at doses above 10 mg. Once-daily dosing of GS-5885 was supported by a long median apparent plasma half-life that ranged from 22 to 50 h. All doses above 3 mg produced a >3 $\log_{10}$ IU mL^{-1} decline in viral load and suppression was more sustained for G-1b-infected patients, although the median maximum reductions were similar for both genotypes 1a and 1b.[81] Of the 72 patients enrolled in this study, four G-1a subjects and one G-1b subject harbored detectable viruses with resistance-associated mutations. Two of the four G-1a-infected patients experienced maximum HCV RNA reductions of <1.6 $\log_{10}$ IU mL^{-1}, of whom one was characterized with a Q30E/Q population and the other harbored L31M virus at baseline. However, one subject dosed with 10 mg of GS-5885 had a maximum viral response of <1.6 $\log_{10}$ and 454 pyrosequencing was necessary to determine that 12% of the virus harbored a Y93C change in the NS5A gene. The emergence of resistance mutations was assessed by population sequencing and all patients dosed at 3 mg or more had virus with detectable changes associated with resistance to GS-5885 *in vitro*. M28T, Q30H, L31M, Q30R and Y93C/H were characterized in G-1a-infected subjects, with Q30R being the most frequent, while Y93H was detected in all 10 G-1b-infected individuals receiving the 10 mg dose of drug. The less resistant M28T and Q30H mutations were not detected in G-1a patients at doses $\geq$30 mg, reflective of plasma concentrations at trough that were above the protein binding-adjusted EC_{90} values.

The quinazoline derivative AZD-2836 (**18**) was the first HCV NS5A inhibitor actually to enter clinical trials, in early 2007, but was abandoned, apparently due to inadequate exposure that was not solved by optimization of the formulation.[27] The antiviral activity of AZD-7295 (**20**) was explored in treatment-naive and treatment-experienced patients infected with genotypes 1a, 1b and 3a virus who were administered the drug for 5 days at doses of 90 and 233 mg tid or 350 mg bid.[27,82] The 90 mg tid cohort comprised five each of G-1a- and G-1b-infected subjects, with the G-1b patients receiving active drug experiencing a mean viral load reduction of over 1 $\log_{10}$ IU mL^{-1}. The higher doses of AZD-7295 (**20**) produced a more pronounced reduction in viral load, up to a maximum of 2.4 $\log_{10}$ IU mL^{-1}, although 4 G-1b patients across the cohorts showed no response to the drug. In contrast, the viral load in G-1a-infected subjects was not statistically significant from placebo, reflecting the poor *in vitro* potency of the compound towards G-1a and a C_{24} drug concentration that did not surpass the EC_{90} measured in the replicon assay. Similarly, AZD-7295 (**20**) at 233 mg tid exerted no significant effect on viral load in the G-3a-infected subjects.

The early clinical studies with HCV NS5A inhibitors revealed a class of antivirals that is characterized by high *in vitro* potency, inhibition of multiple genotypes and excellent efficacy at low doses. The first-generation