rate of which is estimated to range from 0.1 to 1 nucleotide per RNA synthesized.[58–63]

These statistics contribute to the significant population of viruses harboring single (87×10^9 virions per day), double (4.2×10^9 virions per day) and triple (0.13×10^9 virions per day) mutations produced in an infected individual every day.[58–63] Indeed, in a multiple ascending dose study with **1** administered at doses of 1, 10, 30, 60 and 100 mg qd and 30 mg bid for 14 days to chronically infected G-1-infected subjects, the viral load was rapidly reduced by 2.8–4.1 $\log_{10}$ IU mL^{-1} across the cohorts, but most patients experienced rebound on or before day 7 of the treatment period.[64] Viral rebound was associated with the emergence of resistant variants with major substitutions identified at residues M28T/A/V, Q30H/R/K/E, L31M/V and Y93H/C/N for G-1a-infected subjects and L31M/V and Y93H/C for G-1b.[65] These mutant viruses were also observed *in vitro* in G-1a and G-1b replicons placed under selective pressure from **1**. One patient in the 60 mg qd cohort, all of whom were infected with G-1a, had a Q30R mutation detectable at baseline and experienced initial viral suppression, but viral breakthrough occurred by day 14 with a Q30H and Y93H linkage detected that *in vitro* exhibited high resistance to **1**.[65] For two additional patients, Q30E and Y93N variants were detected at day 14, a double mutant associated with high resistance to the drug. The final patient in this cohort experienced failure of therapy with a Q30R virus that emerged within 12 h of drug dosing, despite the fact that the plasma exposure of **1** at day 14 (117 nM) substantially exceeded the *in vitro* replicon EC_{50} of 7 nM.[65] A closer analysis revealed a baseline E62D polymorphism that by itself did not confer resistance to **1** but, when linked to Q30R, conferred high levels of resistance *in vitro*.[66]

Analysis of a cohort of 78 HIV-HCV co-infected subjects and 635 NS5A sequences deposited in the Los Alamos database for the occurrence of baseline resistant mutations to **1** revealed an absence in G-1a and G-3 whereas all G-4 sequences had L31M; the double mutant L31M+Y93H occurred in 7% of G-1b and 13% of G-4 sequences.[67] In a cohort of Japanese subjects infected with G-1b, overwhelmingly the most prevalent in that population, resistance-conferring amino acid substitutions were detected in 11.2% of 294 patients, with Y93H (8.2%) predominating over L31M (2.7%).[68–70]

Taken together, these observations emphasize the anticipation based on virus replication kinetics that combination therapy will be required, either by adding a direct-acting antiviral agent (DAA) to interferon-α/ribavirin therapy or by combinations of DAAs with orthogonal patterns of resistance, to suppress the virus effectively and durably.[61–63,71–75]

In a Phase 2a clinical trial of **1** in conjunction with PEG interferon-α/ribavirin (PEG-IFN/RBV), doses of 3, 10 and 60 mg of the drug were compared with a placebo control arm over a 48 week time span.[76] The primary efficacy endpoint focused on an extended virological response (eRVR), which is defined as undetectable levels of viral RNA at both weeks 4 and 12 after initiation of therapy. Secondary endpoints were rapid virological response (RVR; HCV RNA undetectable at 4 weeks), complete early virological response (cEVR; HCV RNA undetectable at 12 weeks) and sustained virological response at 12