<table>
<tr><td><b>Initiation Codon</b></td><td colspan="3"><b>PVS1 may be applied to initiation codon variants</b></td><td>PVS1</td></tr>
<tr><td rowspan="4"><b>Nonsense or Frameshift</b></td><td colspan="3"><b>Predicted to undergo NMD</b><br>(Premature truncation codon (PTC) NOT in exon 11 or in the 3’-most 50nt of exon 10)<br><b>Nonsense</b> upstream of p.Lys351<br><b>Frameshift</b> induced premature termination codon (PTC) upstream of p.Lys351</td><td>PVS1</td></tr>
<tr><td rowspan="3"><b>Not predicted to undergo NMD</b><br><b>Nonsense</b> downstream of p.Leu350<br><b>Frameshift</b> induced premature truncation codon (PTC) in exon 11 or in the 3’-most 50nt of exon 10.</td><td rowspan="3">Role of region in protein function is unknown</td><td><b>Variant removes >10% of protein</b><br>Nonsense (or frameshift induced PTC) located in the p.Lys351-p.Ala355 range</td><td>PVS1_Strong</td></tr>
<tr><td><b>Variant removes <10% of protein</b><br>Nonsense (or frameshift induced PTC) located in the p.Gly356-p.Asp393 range</td><td>PVS1_Moderate</td></tr>
<tr><td><b>Frameshift induced</b> PTC downstream of the natural stop codon (stop codon read-through)</td><td>PVS1_Moderate</td></tr>
<tr><td rowspan="5"><b>GT--AG 1,2 splice sites</b> (Please see <i>TP53</i> PVS1 Splicing Worksheet for additional SpliceAI scores and information)</td><td colspan="3"><b>Predicted splicing alterations do not affect reading-frame</b><br><b>E1 donor site</b> (Δ1q81)<br><b>E2 acceptor site</b> (Δ2p7)</td><td>PVS1_N/A</td></tr>
<tr><td colspan="3"><b>Predicted splicing alteration targets start codon. Closest potential start codon at p.Met40 (exon 4)</b><br><b>Exon 2 donor</b> (Δ2)</td><td>PVS1</td></tr>
<tr><td colspan="3"><b>Predicted splicing alterations are PTC-NMD (or in-frame targeting critical domains/residues)</b><br><b>E3 acceptor</b> Δ(E3) and/or ▼I2<br><b>E3 donor</b> Δ(E3), ▼I3 and/or ▼I3<br><b>E4 acceptor</b> Δ(E4p19) and/or ▼I3 and/or Δ(E4)<br><b>E4 donor</b> Δ(E4q200), ▼I4, Δ(E4q62) and/or ▼(E4q391)<br><b>E5 acceptor</b> ▼I4 and/or Δ(E5p21)<br><b>E5 donor</b> Δ(E5q46) and/or ▼I5<br><b>E6 acceptor</b> Δ(E6p17), Δ(E6), Δ(E6p1) and /or ▼I5<br><b>E6 donor</b> ▼(E6q5), Δ(E6), ▼I6 and/or▼(E6q18)<br><b>E7 acceptor</b> ▼(E7p49), Δ(E7) and/or ▼I6<br><b>E7 donor</b> ▼I7 and/or ▼(E7q1), ▼(E7q290) and/or Δ(E7)<br><b>E8 donor</b> Δ(E8) and/or ▼I8<br><b>E9 acceptor</b> Δ(E9p19), Δ(E9) and/or ▼I8<br><b>E9 donor</b> ▼(E9q328), Δ(E9) and/or▼I9<br><b>E10 acceptor</b> ▼(E10p44) and/or ▼I9</td><td>PVS1</td></tr>
<tr><td colspan="3"><b>Some predictions are very short in-frame with no obvious functional impact</b><br><b>E8 acceptor</b> ▼I7, Δ(E8p24), and/or ▼(E8p3)</td><td>PVS1_N/A</td></tr>
<tr><td colspan="3"><b>Splicing alteration is predicted to shorten (<10% of protein removed) or expand the C-terminal end of unknown function</b><br><b>E10 donor</b> ▼I10<br><b>E11 acceptor</b> ▼I10 and/or Δ(E11p10)</td><td>PVS1_Moderate</td></tr>
</table>

| | | | | |
|---|---|---|---|---|
| **Deletion**<br>(Single exon to full gene) | Full gene deletion | | | PVS1 |
| | Single exon deletion does not target the coding sequence | | Exon is present in biologically-relevant transcript NM_000546.6<br>**delE1** | PVS1_N/A |
| | Single to multi exon deletion that targets the initiation codon, preserving the potential rescue ATG (p.Met40) in exon 4 | | Exon is present in biologically-relevant transcript NM_000546.6<br>Multi-exon combinations starting upstream of exon 3<br>**delE1_E2, delE1_E3, delE2, delE2_E3** | PVS1 |
| | Single to multi exon deletion that targets the initiation codon and the potential rescue ATG (p.Met40) in exon 4 | | Exon is present in biologically-relevant transcript NM_000546.6<br>Multi-exon combinations starting upstream of exon 3<br>**(e.g. delE1_E7, delE2_E5, delE2_E10)** | PVS1 |
| | Single to multi exon deletion that disrupts the reading frame and is predicted to undergo NMD<br>(Nonsense or frameshift induced PTC upstream of p.Lys351) | | Exon is present in biologically-relevant transcript NM_000546.6<br>Single. Only possibilities are:<br>**delE3, delE5, delE6, delE7, delE8, delE9**<br>Multi-exon combinations. Only possibilities are:<br>**delE3_E4, delE3_E5, delE3_E6, delE3_E8, delE3_E9**<br>**delE4_5, delE4_E7, delE4_8E, delE4_E9**<br>**delE5_E7, delE5_E8**<br>**delE6_E7, delE6_E9**<br>**delE7_E8** | PVS1 |
| | Single to multi exon deletion that disrupts the reading frame and is **NOT** predicted to undergo NMD<br>(Nonsense or frameshift induced PTC downstream of p.Leu350) | | Truncated/altered region is critical to protein function<br>Single<br>**delE10**<br>Multi-exon combinations ending in intron 10. Only possibilities are:<br>**delE4_E10, delE5_E10, delE6_E10, delE7_E10, delE9_E10** | PVS1 |
| | Single to multi exon deletion includes last exon | | Truncated/altered region is critical to protein function<br>(Any multi-exon combinations targeting exon 11)<br>**(e.g. delE10_E11 , delE5_E11)** | PVS1 |
| | | Role of region in protein function is unknown | Variant removes <10% of protein<br>**delE11** | PVS1_Moderate |
| | Single to multi exon deletion preserves the reading-frame | | Truncated/altered region is critical to protein function<br>Single<br>**delE4**<br>Multi-exon combinations. Only possibilities are:<br>**delE3_E7, delE3_E10, delE4_E6, delE4_E9, delE5_E6, delE5_E9, delE6_E8, delE7_E9, delE8_E10** | PVS1 |
| **Duplication**<br>(≥1 exon in size and must be completely contained within the *TP53* gene) | Proven in tandem | | Single to multi exon duplication that targets the initiation codon (i.e. 5' breakpoint in intron 1)<br>**(e.g. dupE2, dupE2_E3, dupE2_E4, dupE2_E5, …, dupE2_E10)** | PVS1_N/A |
| | | | Reading frame disrupted and NMD predicted to occur<br>(Nonsense upstream of p.Lys351 or frameshift-induced PTC upstream of p.Lys351)<br>**(e.g. dupE3, dupE5_E7)** | PVS1 |
| | | | No or unknown impact on reading frame and NMD<br>**(e.g. dupE4, dupE5_E6)** | PVS1_N/A |
| | Presumed in tandem | | Single to multi exon duplication that targets the TP53 initiation codon (i.e. 5' breakpoint in intron 1)<br>**(e.g. dupE2, dupE2_E3, dupE2_E4, dupE2_E5, …, dupE2_E10)** | PVS1_N/A |
| | | | Reading frame presumed disrupted and NMD predicted to occur<br>(Nonsense upstream of p.Lys351 or frameshift-induced PTC upstream of p.Lys351)<br>**(e.g. dupE3, dupE5_E7)** | PVS1_Strong |
| | | | No or unknown impact on reading frame and NMD<br>**(e.g. dupE4, dupE5_E6)** | PVS1_N/A |
| | Proven not in tandem | | | PVS1_N/A |

**Figure 1. Flowchart for the application of PVS1 to *TP53* null variants in relation to the transcript NM_000546.6.** Splicing predictions for GT-AG sites are based on SpliceAI and available experimental data. PVS1_Variable Weight (RNA) may be applicable for variants with RNA-based assay data demonstrating aberration (see Supplementary Table S1). Δ = exon skipping, ▼= intron retention