Protein sequences:

>P53_HUMAN Cellular tumor antigen p53 - Healthy Tissue MEEPQSDPSVEPPLSQETFSDLWKLLPENNVLSPLPSQAMDDLMLSPDDIEQWFTEDPGP DEAPRMPEAAPPVAPAAAPTPAAPAPAPSWPLSSSVPSQKTYQGSYGFRLGFLHSGTAK SVTCTYSPALNKMFCQLAKTCPVQLWVDSTPPPGTRVRAMAIYKQSQHMTEVVRRCPHHE RCSDSDGLAPPQHLIRVEGNLRVEYLDDRNTFRHSVVVPYEPPEVGSDCTTIHYNYMCNS SCMGGMNRRPILTIITLEDSSGNLLGRNSFEVRVCACPGRDRRTEEENLRKKGEPHHELP PGSTKRALPNNTSSSPQPKKKPLDGEYFTLQIRGRERFEMFRELNEALELKDAQAGKEPG GSRAHSSHLKSKKGQSTSRHKKLMFKTEGPDSD

>P53_HUMAN Cellular tumor antigen p53 - Tumor Tissue MEEPQSDPSVEPPLSQETFSDLWKLLPENNVLSPLPSQAMLDLMLSPDDIEQWFTEDPGPDEAPWMPEAAPPVAPAAPTPAAPAPAPAPSWPLSSSVPSQKTYQGSYGFRLGFLHSGTAKSVTCTYSPALNKMFCQLAKTCPVQLWVDSTPPPGTRVRAMAIYKQSQHMTEVVRRCPHHERCSDSDGLAPPQHLIRVEGNLRVEYLDDRNTFVHSVVVPYEPPEVGSDCTTIHYNYMCNSSCMGGMNRRPILTIITLEV

Patient HLA typing results:

HLA-A*02:01

HLA-A*68:01

HLA-B*07:02

HLA-B*35:01

Step 1: Mutated regions identified

>D41L

SPLPSQAMLD**L**MLSPDD

>R65W

DPGPDEAP**W**MPEAAPPV

>R213V

YLDDRNTF**V**HSVVVPYE

>D259V

ILTIITLEV

Step 2: HLA binding predictions on IEDB

Allele	#	Start	End	Length	Peptide	Method used	Percentile_rank
HLA-A*02:01	3	1	9	9	YLDDRNTFV	Consensus (ann/comblib_sidney2008/smm)	0.2

HLA-B*35:01	3	8	16	9	FVHSVVVPY	Consensus (ann/comblib_sidney2008/smm)	0.2
HLA-B*07:02	1	1	9	9	SPLPSQAML	Consensus (ann/comblib_sidney2008/smm)	0.4
HLA-A*02:01	2	9	17	9	WMPEAAPPV	Consensus (ann/comblib_sidney2008/smm)	0.4
HLA-B*07:02	1	3	11	9	LPSQAMLDL	Consensus (ann/comblib_sidney2008/smm)	0.5
HLA-A*02:01	4	1	9	9	ILTIITLEV	Consensus (ann/comblib_sidney2008/smm)	0.7
HLA-A*68:01	3	8	16	9	FVHSVVVPY	Consensus (ann/smm)	2.1
HLA-B*35:01	1	3	11	9	LPSQAMLDL	Consensus (ann/comblib_sidney2008/smm)	2.2
HLA-B*35:01	2	7	15	9	APWMPEAAP	Consensus (ann/comblib_sidney2008/smm)	2.2
HLA-B*07:02	2	7	15	9	APWMPEAAP	Consensus (ann/comblib_sidney2008/smm)	2.8
HLA-A*02:01	1	7	15	9	AMLDLMLSP	Consensus (ann/comblib_sidney2008/smm)	4
HLA-B*35:01	2	6	14	9	EAPWMPEAA	Consensus (ann/comblib_sidney2008/smm)	4.2
HLA-B*35:01	1	1	9	9	SPLPSQAML	Consensus (ann/comblib_sidney2008/smm)	5.2
HLA-B*35:01	2	1	9	9	DPGPDEAPW	Consensus (ann/comblib_sidney2008/smm)	5.2

Step3: BLAST identified top binding peptides to check if they are encoded in other human proteins?

Blastp Input:

```
>HLA-A0201_top_pep
YLDDRNTFV
> HLA-A6801_HLA-B3502_top_pep
FVHSVVVPY
>HLA-B0702_top_pep
SPLPSQAML
```

→ All three have top hit in p53 sequence of the human proteome with 8/9 residues matching

Final question: Which peptide would you choose?

Probably FVHSVVVPY, Better yet YLDDRNTFVHSVVVPYE