Question 1:

A major advantage of CRISPR over traditional programmable designer nucleases is:

- CRISPR's reliance on sgRNA as the targeting mechanism.

  Explanation:

  sgRNA can be quickly and cheaply redesigned to target a new genomic site, whereas ZFNs and TALENs require whole-protein engineering to target new loci. Additionally, multiple sgRNAs can be delivered to target multiple genes simultaneously.

❍ CRISPR's higher gene targeting efficiency.

❍ CRISPR's lower off-targeting efficiency.

Question 2:

HDR is less efficient than NHEJ because:

❍ HDR is inherently mutagenic and bad for the host.

- HDR relies on endogenous cellular proteins involved in homologous recombination.

  Explanation:

  HDR relies on a homologous donor DNA template to direct a precise DSB repair. This choice is correct because HDR relies on endogenous proteins involved in homologous recombination that are expressed during S and G2 phases of the cell cycle. Choice #1 is wrong because HDR is seldom mutagenic or bad for the host, unless the homologous donor contains a lethal mutation. Choice #3 is wrong because HDR does not rely on sequence-specific motifs.

❍ HDR relies on particular sequence-specific motifs that reduce its frequency, whereas NHEJ does not.

Question 3:

Off-target mutations are a result of:

❍ Mutant nuclease variants that randomly cut and mutate the genome.

❍ Flawed sgRNA design, which hyper-activates Cas9 nuclease activity to randomly cut and mutate non-targeted sites.

● Non-targeted sites sharing sequence homology with the targeted locus.

Explanation:

This answer is correct because off-target mutations occur as a result of the designer nucleases honing in on a site sharing homology with the targeted site. Choices 1 & 2 are incorrect because off-target mutations do not occur at random genomic sites. They occur at sites that share sequence homology with the targeted site.

Question 4:

CRISPR-mediated genome-wide screens rely on _____ to target _____.

● sgRNA libraries; multiple genes simultaneously

Explanation:

The sgRNA honing mechanism allows for multiple genes to be targeted simultaneously because sgRNAs are relatively small (~100bp), can be cheaply produced, and pooled together to form a sgRNA library targeting hundreds to thousands of genes. The second choice is wrong because the lentiviruses are the delivery mechanism used to introduce the sgRNA library into the melanoma cells. Gene targeting is accomplished by the sgRNA library. The third choice is wrong because, while CRISPR-mediated genome-wide screens do rely on Cas9 to cut the targeted DNA, the CRISPR treatment alone does not result in melanoma cell death.

❍ lentiviruses; gene mutations involved in the acquired resistance to melanoma chemotherapy agents

❍ Cas9; and kill melanoma cells

Question 5:

Cas9 is guided to the targeted locus by:

❍ Endogenous genomic palindromic adjacent motifs.

● sgRNA.

Explanation:

This answer is correct because sgRNA guides Cas9 to the genomic target. Choice #1 is wrong because, while PAM sites are required for gene targeted, the PAM site does not direct the Cas9 to the target. Choice #3 is incorrect because CRISPR genome editing does not involve TALENs.

❍ TALENs.