

## **Figure Legends for Teaching Cases #1-9 (Supplemental Digital Content)**

### **Teaching Case #1- Endometrial curetting, MSH2 deficient**

#### Supplemental Digital Content Figure 1.1:

MLH1 staining (40x magnification). Variable staining of MLH1 is seen with some cytoplasmic staining in the normal epithelium.

#### Supplemental Digital Content Figure 1.2:

MLH1 staining (200x magnification). Variable nuclear MLH1 staining in normal tissue is seen. There is background cytoplasmic staining which can impact interpretation. The tumor tissue shows strong nuclear staining and should be interpreted as MLH1 intact.

#### Supplemental Digital Content Figure 1.3:

MLH1 staining (200x magnification). In areas of cauterization the normal epithelium shows increased cytoplasmic staining and loss of nuclear staining. Areas such as this should be interpreted with caution and other areas of the tumor should be used to assess MLH1 status.

#### Supplemental Digital Content Figure 1.4:

MSH2 staining (40x magnification). Loss of nuclear MSH2 staining in the tumor (MSH2 deficient). The normal epithelium shows cytoplasmic staining which should be ignored.

#### Supplemental Digital Content Figure 1.5:

MSH2 staining (200x magnification). The tumor shows loss of nuclear staining (MSH2 deficient).

#### Supplemental Digital Content Figure 1.6:

MSH2 staining (200x magnification). Areas of normal epithelium can also show loss of nuclear staining.

### **Teaching Case #2- Endometrial biopsy, Normal MMR (variable MLH1 expression)**

#### Supplemental Digital Content Figure 2.1:

MLH1 staining (40x magnification). The majority of the tumor shows strong nuclear staining for MLH1, but there are areas with decreased nuclear intensity.

#### Supplemental Digital Content Figure 2.2:

MLH1 staining (40x magnification). Some large sections of the tumor show less intense staining with MLH1.

#### Supplemental Digital Content Figure 2.3:

MLH1 staining (200x magnification). Higher power shows that there is nuclear staining with MLH1, the intensity is not the same throughout the tumor, but there is no area with loss of MLH1 expression. The tumor is microsatellite stable (MSS) and MLH1 is considered intact.

#### Supplemental Digital Content Figure 2.4:

MLH1 staining (200x magnification). The variability of tumor staining can be seen in multiple areas within the biopsy.

Supplemental Digital Content Figure 2.5:

MSH2 staining (200x magnification). MSH2 shows strong nuclear staining but there is also cytoplasmic staining which can make interpretation difficult.

**Teaching Case #3-Hysterectomy, Normal MMR**

Supplemental Digital Content Figure 3.1 (a-b):

MLH1 and (b) MSH6 staining (40x magnification). Staining is faint in both normal and tumor tissue. As a result, care must be taken to ensure the tumor is not over-interpreted as having absent expression of MLH1 or MSH6.

Supplemental Digital Content Figure 3.2:

MLH1 and (d) MSH6 (200x magnification). Faint staining seen in both tumor and normal tissue.

**Teaching Case #4-Hysterectomy, Normal MMR**

Supplemental Digital Content Figure 4.1:

MSH6 staining (40x magnification). MSH6 often shows more staining variability than other immunohistochemical staining. There is variable staining within the tumor cells of this tissue.

Supplemental Digital Content Figure 4.2:

MSH6 staining (200x magnification). MSH6 staining is variable within the tumor. There is a discrete area with lack of MSH6 expression. The case is microsatellite stable (MSS) and the tumor should be considered MSH6 intact.

**Teaching Case #5-Hysterectomy, Normal MMR (variable MSH2 and MSH6 expression)**

Supplemental Digital Content Figure 5.1 (a-b):

MSH2 staining (40x magnification). Some regions of the tumor show variable staining intensity. When one area of the tumor shows faint staining, the remainder of the block should be examined to assess the tumor status (b). MSH6 showed a similar pattern. This case is MSH2 / MSH6 intact.

Supplemental Digital Content Figure 5.2 (a-b):

MSH2 staining (200x magnification). The tumor shows a region of weaker nuclear staining than seen in the majority of the tumor (c). The case is MSH2 intact.

**Teaching Case #6-Hysterectomy, MSH6 deficient**

Supplemental Digital Content Figure 6.1:

MSH6 staining (40x magnification). The tumor shows a lack of nuclear MSH6 expression (MSH6 deficient). There is patchy cytoplasmic staining which makes assessment difficult. In cases with intense cytoplasmic or membranous staining, careful investigation of the tumor is needed to ensure that the nuclear staining can be clearly assessed.

Supplemental Digital Content Figure 6.2:

MSH6 staining (200x magnification). The tumor shows intense cytoplasmic staining which makes assessment of the nuclear staining difficult. There is no nuclear expression and the tumor should be considered MSH6 deficient.

Supplemental Digital Content Figure 6.3:

MSH6 staining (200x magnification). There are areas of the tumor with no nuclear or cytoplasmic staining making assessment difficult. Given the strong staining in the stromal cells, focal rare, weak staining in the tumor is not significant. The tumor should be considered MSH6 deficient.

**Teaching Case #7-Colon resection, MSH6 deficient**

Supplemental Digital Content Figure 7.1:

MSH6 staining (40x magnification). The vast majority of the tumor shows absent nuclear expression of MSH6 (MSH6 deficient). There are focal areas which show tumor staining. Staining is seen in <10% of the tumor cells and the case should be considered MSH6 deficient.

Supplemental Digital Content Figure 7.2:

MSH6 staining (200x magnification). Higher power showing focal staining of MSH6. There is clear absent expression within the majority of the tumor and strong nuclear staining in the adjacent stromal cells. The case is MSH6 deficient.

Supplemental Digital Content Figure 7.3 (a-b):

MSH2 staining (40x magnification (a) / 200x magnification (b)). There is variable MSH2 staining but the majority of the tumor shows strong nuclear staining. This variability can be seen in some cases with abnormal MSH6 expression. The tumor is MSH2 intact.

**Teaching Case #8-Endometrial curetting, MLH1 deficient**

Supplemental Digital Content Figure 8.1 (a-b):

MLH1 staining (40x magnification). The tumor shows variable MLH1 expression. There are clear areas with abnormal staining, while other areas show nuclear expression. While case 7 showed only focal tumor staining, this case shows an area of tumor with clear expression, while the majority of the tumor shows abnormal expression. Given the amount of tumor with expression is <10% of the tumor the case should be considered MLH1 deficient.

Supplemental Digital Content Figure 8.2 (a-b):

MLH1 staining (200x magnification). Higher power magnification showing areas of tumor with abnormal expression and normal expression (b).

Supplemental Digital Content Figure 8.3:

MLH1 staining (200x magnification). Areas of squamous metaplasia within the tumor also show abnormal MLH1 expression.

**Teaching Case #9- Right hemicolectomy, abnormal MLH1 expression**

Supplemental Digital Content Figure 9.1:

MLH1 staining (40x magnification). The tumor shows abnormal MLH1 expression (MLH1 deficient) with focal weak tumor staining.

Supplemental Digital Content Figure 9.2 (a-b):

MLH1 staining (200x magnification). There are large areas of the tumor with weak MLH1 expression (A) in tumor compared to the adjacent stroma. Given the absent areas of expression (B) with other areas showing only weak staining, the case is considered to have abnormal MLH1 expression.