SnapshotDx Quiz: January 2020

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**WHAT IS YOUR DIAGNOSIS?**

![Image](image_url)

*Figure 1.* Image courtesy of Milan J. Anadkat, Washington University School of Medicine in St. Louis.

*Editorial note: Welcome to the Journal of Investigative Dermatology (JID) Snapshot Dx Quiz. In this monthly online-only quiz, the first question ("What is your diagnosis?") relates to the clinical image shown, while additional questions concern the findings reported in the JID article by Koopmans et al. (2019) ([https://doi.org/10.1016/j.jid.2019.01.038](https://doi.org/10.1016/j.jid.2019.01.038)). Detailed answers and a list of relevant references are available following the Quiz Questions below.

**QUIZ QUESTIONS**

1. **What is your diagnosis?**
   a. Compound melanocytic nevus
   b. Melanoma
   c. Spitz nevus
   d. Blue nevus
   e. Pigmented basal cell carcinoma

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2. **Which of the following statements is TRUE?**
   a. UV exposure results in cutaneous pigmentation when stimulation of melanocortin 1 receptor (MC1R) leads to secretion of alpha-melanocyte stimulating hormone (MSH) in epidermal melanocytes.
   b. Microphthalmia-associated transcription factor (MITF) amplification occurs in approximately 80% of melanomas.
   c. NRAS mutations are the most common oncogenic driver of melanoma.
   d. MAPK/ERK kinase (MEK) inhibitor therapy, in conjunction with BRAF-inhibitor therapy, is appropriate for patients with BRAF-mutant invasive melanoma.
   e. Melanoma patients treated with BRAF-inhibitor therapy rarely experience disease progression after treatment.

3. **According to the article by Koopmans et al. (2019), which of the following is TRUE regarding the current PD-L1 inhibiting immune therapy?**
   a. Causes indiscriminate activation of naïve T-cells
   b. Is rarely associated with adverse events owing to tumor cell selectivity
   c. Causes upregulation of T-cell production through the division of mature T-cells
   d. Binds “on-target” and “off-tumor” leading to optimal efficacy
   e. Promotes T-cell proliferation and the production of granzyme B and IFN-γ

4. **According to the article by Koopmans et al. (2019), which of the following is TRUE regarding the bispecific antibody, PD-L1xCSPG4?**
   a. May enhance binding affinity (avidity) compared with the standard PD-L1 therapy in 10% of melanomas
   b. Its PD-L1 blocking ability was always superior compared with the standard PD-L1 therapy
   c. Can bind to two different cell types simultaneously
   d. Did not result in an increase in apoptotic cancer cell death compared with the control
   e. Its PD-L1 blocking activity was high regardless of cancer cell CSPG4-expression

See following pages for detailed answers.
DETAILED ANSWERS

1. What is your diagnosis?

CORRECT ANSWER: b. Melanoma

Malignant melanoma is associated with relatively high mortality, and its incidence is increasing (MacKie et al., 2009). Risk factors for melanoma include: UV exposure (especially tanning bed use <30 years of age; history of blistering sunburn(s); history of significant childhood sun exposure), first degree relative with melanoma, blonde or red hair or blue eyes, >100 nevi, and ≥3 atypical nevi. The clinical appearance of melanoma is often dependent on subtype. For example, superficial spreading melanoma (representing ~70% of all melanomas) is macular or a thin plaque with brown-black color variation and irregular borders, as depicted by Fitzpatrick and Wolff (2008). After wide excision, early-stage disease (<4 mm Breslow depth and node-negative) does not require adjuvant therapy (Bologna et al., 2018). However, mortality and metastatic potential increase with increasing depth of invasion (Cecil et al., 2012). For node-positive melanomas, or those >4mm in thickness, postsurgical adjuvant therapy is recommended (Coit et al., 2019).

Immunotherapy and targeted therapy (PD-1 or PD-L1, CTLA-4, BRAF, and MEK inhibitors) offer improved progression-free and overall survival rates compared with previous therapies. The median overall survival of patients with disseminated melanoma has increased from 9 months to at least 2 years (Cecil et al., 2012).

Discussion of incorrect answers

a. Compound melanocytic nevus: Compound nevi are the most common type of melanocytic nevi. Histologically, nests of melanocytes can be seen at the dermal-epidermal junction and in the dermis. On inspection, they are generally lighter in color (light to medium brown for light-skinned individuals) than junctional nevi or melanomas. Furthermore, unlike the lesion photographed, they are usually well-circumscribed and symmetric.

c. Spitz nevus: This uncommon nevus typically presents as a sharply circumscribed, dome-shaped papule in the pediatric population. Although their color is variable (may be black), they are generally pink to red-brown. Usually, they do not meet the ABCDE criteria (asymmetry, border irregularities, color variation, dimensions or diameter, evolution or change). However, microscopically, they appear very similar to melanoma.

d. Blue nevus: Blue nevus generally presents as a uniformly blue or blue-black, dome-shaped papule. Notably, skin markings over the nevus are preserved, and blue nevi usually do not demonstrate atypical features.

e. Pigmented basal cell carcinoma: Basal cell carcinoma (BCC) is the most common malignancy, with pigmented subtype representing the minority of lesions in fair-skinned individuals (approximately 6%), and is more common in individuals with darker skin. A pigmented BCC often presents as a sharply demarcated, dark brown-black nodule, sometimes demonstrating ulceration.

2. Which of the following statements is TRUE?

CORRECT ANSWER: d. MEK inhibitor therapy in conjunction with BRAF-inhibitor therapy, is appropriate for patients with BRAF-mutant invasive melanoma.

In the RAS/RAF pathway of cellular signaling, BRAF mutations result in activation of MEK (and subsequently extracellular signal-regulated kinase) and lead to oncogenesis through excessive cell proliferation and opposition of apoptosis. Therefore, the presence of BRAF mutation (V600E being the most common) correlates strongly with response to MEK inhibitor therapy (Solit et al., 2006). For patients with BRAF-mutant metastatic melanoma, clinical trials of BRAF inhibitor therapy (e.g., trametinib + dabrafenib) have demonstrated prolonged survival compared with BRAF therapy alone (Long et al., 2017).

Discussion of incorrect answers

a. UV exposure results in cutaneous pigmentation when stimulation of MC1R leads to secretion of MSH in epidermal melanocytes: The mechanism by which UV exposure induces cutaneous pigmentation is through keratinocyte DNA damage stimulating p33-mediated induction of proopiomelanocortin or MSH expression (Cui et al., 2007). MSH secretion by epidermal keratinocytes stimulates MC1R on melanocytes (Lin and Fisher, 2007). MC1R gene variants produce red hair and fair skin phenotypes in individuals who, as a result, have an increased risk of melanoma and non-melanoma skin cancers (Robles-Espinoza et al., 2016; Valverde et al., 1995). MC1R is stimulated with similar efficacy by MSH and by adrenocorticotropin hormone, which explains the classic hyperpigmentation seen in Addison’s disease (Carlson et al., 2007).
b. MITF amplification occurs in approximately 80% of melanomas: Stimulation of MC1R results in downstream expression of MITF, which then contributes to the production of melanin pigment. MITF amplification occurs in approximately 20% of melanomas and is generally associated with a poor prognosis (Garraway et al., 2005; Ugurel et al., 2007).

c. NRAS mutations are the most common oncogenic driver of melanoma: BRAF mutations are the most common genetic abnormality in cutaneous melanoma, implicated in up to 66% of melanoma cases (Davies et al., 2002). NRAS mutations are the second most common genetic abnormality, present in approximately 15–20% of melanoma cases (van ‘t Veer et al., 1989). Melanomas with NRAS mutations are more aggressive and associated with poorer outcomes (Ball et al., 1994). The treatment landscape for BRAF-mutant melanoma has evolved significantly with immune checkpoint inhibitors and molecular-targeted therapies. Immune checkpoint inhibitors have demonstrated efficacy in NRAS mutated melanomas (Johnson et al., 2015). The development of targeted NRAS inhibitors has proven problematic; however, these agents are an area of active investigation (Yin et al., 2019). Because NRAS activating mutations also activate the mitogen-activated protein kinase (MAPK) pathway, MEK inhibitors have been used with some success (Ascierto et al., 2013).

e. Melanoma patients treated with BRAF-inhibitor therapy rarely experience disease progression after treatment: Unfortunately, most patients treated with a BRAF-inhibitor will eventually develop resistance to therapy, and the disease will subsequently progress (Flaherty et al., 2010). Although a universal mechanism of resistance has not been identified, it is thought to be related to alternate pathways of activation of the MAPK pathway (Nazarian et al., 2010). Notably, primary resistance to BRAF-inhibitor therapy (demonstrated by disease progression during initial therapy) is rare (<10% of patients treated with vemurafenib) (Flaherty et al., 2010).

3. According to the article by Koopmans et al. (2019), which of the following is TRUE regarding the current PD-L1 inhibiting immune therapy?

CORRECT ANSWER: e. Promotes T-cell proliferation, and the production of granzyme B and IFN-γ

In this study by Koopmans et al. (2019), MEDI4736 (Durvalumab: a currently FDA-approved PD-1 inhibitor) was used as a comparator in assessing the efficacy of a novel bispecific antibody to both PD-L1 and CSPG4 (chondroitin sulfate proteoglycan 4: selectively overexpressed in several cancer types including melanoma). The authors evaluated the effectiveness of MEDI4736 and PD-L1xCSPG4 by measuring autologous T-cells’ capacity to proliferate (via T-cell count), secrete IFN-γ, and secrete granzyme B (Figure 3a–d) after treatment. The authors show that both MEDI4736 and PD-L1xCSPG4 enhance the activation status of antigen-experienced T-cells.

Discussion of incorrect answers

a. Causes the indiscriminate activation of naïve T-cells: Antigen-presenting cells provide positive and negative costimulatory signals to regulate the immune response of T-cells. While naïve T-cells are limited to the lymph nodes and circulatory system, T-cells which have been activated by an antigen-presenting cell are “antigen-experienced” and express PD-1 receptors. In other words, PD-1 expression on T-cells is induced upon T-cell receptor activation (Chikuma et al., 2009). Since current immunotherapies target PD-1 receptors without specificity or discrimination, all antigen-experienced T-cells are potentially affected. Therefore, PD-L1 inhibiting immune therapy causes indiscriminate activation of antigen-experienced T-cells. In this paper, the authors used T-cells derived from cytomegalovirus seropositive patients as antigen-experienced T-cells.

b. Is rarely associated with adverse events owing to tumor cell selectivity: Given that PD-1 and PD-L1 therapies lack intrinsic tumor specificity, they act non-selectively at PD-1 receptors on T-cells or PD-L1 receptors on dendritic cells. Therefore, these therapies can be associated with significant autoimmune-related adverse events. PD-1 inhibitor adverse events were summarized in one systematic review and included: hypothyroidism, pneumonitis, colitis, hypophysitis, and rash (Baxi et al., 2018).

c. Causes the upregulation of T-cell production through the division of mature T-cells: Although the mechanism by which mature individuals develop new T-cells is via division of mature T-cells outside of the central lymphoid organs (Janeway, 2001), this is not the mechanism by which PD-L1 inhibitors act. Instead, PD-L1 inhibitors’ anticancer effects are a result of functionally-impaired T-cell reactivation. The “programmed death-1” receptor (PD-1 on T-cells and its ligand PD-L1 on dendritic cells) is an immunologic “brake”. The binding of PD-L1 on tumor cells to PD-1 on T-cells leads to impaired signaling and CD28 cosimulation.
Therefore, PD-1/PD-L1 inhibiting therapies “release the brake” and allow for T-cells to function.

d. **Bind “on-target” and “off-tumor” leading to optimal efficacy:** Present PD-L1 inhibitors lack intrinsic tumor selectivity, and therefore bind to any cell expressing PD-L1. PD-L1 binding to non-tumor cells is “on-target” but “off-tumor” and may actually lead to reduced efficacy. The non-tumor PD-L1 receptors may essentially dilute the effect of the therapeutic antibody by precluding sufficient accumulation at the tumor site. In addition, PD-L1 is expressed in some rare normal cells (Dong et al., 1999).

4. According to the article by Koopmans et al. (2019), which of the following is TRUE regarding the bispecific antibody, PD-L1xCSPG4?

**CORRECT ANSWER: c. Can bind to two different cell types simultaneously**

Over 90% of melanoma tumors express CSPG4 (Campoli et al., 2010). PD-L1xCSPG4 simultaneously binds to PD-L1 and CSPG4 and the concurrent binding increases avidity to PD-L1<sup>POS</sup>/CSPG4<sup>POS</sup> tumor cells. It was also determined that PD-L1xCSPG4 can “bridge” two different cell types that express either PD-L1 and/or CSPG4. The authors state that this unique function adds to the antitumor activity of the antibody by modulating intercellular contacts. This is important because PD-L1xCSPG4 can act locally in blocking the immune suppressive activity of tumor-infiltrating leukocytes that express elevated cell surface levels of PD-L1 to exert this effect.

**Explanation of incorrect answers**

a. **May enhance binding affinity (avidity) compared with the standard PD-L1 therapy in 10% of melanomas:** The authors cite that CSPG4 is overexpressed in up to 90% of melanoma lesions (Campoli et al., 2010). Given the finding that this bispecific antibody is associated with increased avidity (Figure 1), it is possible that this therapy could be more effective than standard PD-L1 therapy for a majority of melanoma patients whose tumors express this protein.

b. **PD-L1 blocking ability was always superior compared with the standard PD-L1 therapy:** In PD-L1-positive, CSPG4-negative tumor cells, PD-L1xCSPG4 actually demonstrated less PD-L1 blocking activity than the standard PD-L1 therapy. The authors hypothesize that this is because of the molecular link within the bispecific antibody reducing accessibility to either or both target antigens. However, the PD-L1 blocking activity of PD-L1xCSPG4 was notably higher than standard PD-L1 therapy in PD-L1-positive, CSPG4-positive tumor cells. Therefore, improved PD-L1 blocking activity required concurrent binding to cancer cell surface-expressed CSPG4.

d. **Did not result in an increase inapoptotic cancer cell death:** The authors isolated melanoma cells from five individual patient tumors. All tumors expressed cell surface PDL-1 and CSPG4 over the control levels. Tumor-infiltrating lymphocytes derived from melanoma patients were then cocultured with their autologous melanoma cells and treated with PD-L1xCSPG4 or control antibodies. Figure 5c demonstrates up to a 25% increase in apoptotic cancer cell death compared with control.

e. **PD-L1 blocking activity was high regardless of cancer cell CSPG4-expression:** As described in the explanation for answer choice b, in PD-L1-positive, CSPG4-negative tumor cells, PD-L1xCSPG4 actually demonstrated less PD-L1 blocking activity compared with standard PD-L1 therapy. Improved PD-L1 blocking activity required concurrent binding to cancer cell surface-expressed CSPG4.

**REFERENCES**


