Ancillary studies (Non-core)

BRAF testing

Based on recent advances in our understanding of the molecular basis of melanoma and the role of the immune system in controlling the disease have led to multiple new therapeutic strategies that have radically transformed the care of melanoma patients, particularly those with advanced stage disease. These treatments were initially shown to be effective in patients with stage IV melanoma but more recently have demonstrated a 50% reduction in the rate of relapse for patients with stage III melanoma and are now being trialled in patients with earlier stage melanoma. Examples of these new effective drug therapies approaches are immunotherapy, using immune system checkpoint inhibitors against CTLA-4[1,2] and/or PD-1,[3-5] and molecularly-targeted therapy using BRAF inhibitors alone (monotherapy)[6-10] or in combination with MEK inhibitors[11-15] for the approximately 40-50% of patients with metastatic melanoma whose melanoma harbors a BRAF V600 mutation.[16,17]

BRAF mutations in melanoma are predominantly V600E (73-90%) and V600K (5-20%), but occasionally are other genotypes. There is an inverse relationship between BRAF mutation prevalence and age. Almost all patients <30 years and only 25% of patients ≥70 years had BRAF-mutant melanoma. Amongst BRAF-mutant melanoma, the frequency of non-V600E genotypes (including V600K) increase with increasing age. There are various molecular techniques for detecting BRAF and other somatic gene mutations within melanoma and these techniques are associated with varying sensitivity and specificity. With all techniques, careful macrodissection by pathologists to enrich for tumour cells is usually an important pre-analytical step to ensure optimal results of testing. The presence of BRAFV600E mutation can be detected by immunohistochemistry, but there are as yet no validated antibodies available for the detection of BRAFV600K mutations, and hence alternative techniques are required.

Other ancillary testing

In selected circumstances, molecular ancillary studies can be helpful when evaluating melanocytic tumours. In difficult melanocytic tumours, in which accurate characterization of the tumour as benign or malignant is difficult based on routine histopathology, it may be useful to assess for the presence of chromosomal copy number aberrations.

Comparative genomic hybridization (CGH) and fluorescence in situ hybridization (FISH) can be used to detect chromosomal copy number aberrations in formalin-fixed, paraffin-embedded tissue.[18,19] FISH can be utilized to directly visualize specific chromosomal copy number changes within individual tumour cells. While it has the limitation of being able to test for only a limited number of changes (compared to CGH, which tests for chromosomal aberrations in the entire genome), FISH is more easily applied in routine clinical practice and can be successfully performed on small tumour samples. CGH is generally only available in specialist centres, and is expensive and not applicable to small samples.

Gene expression has also been used to assist in the classification of borderline melanocytic tumours and a number of commercially available tests have been developed utilising this technique. However, these tests need further validation before they can be recommended for routine (i.e., beyond adjunct) use in the clinical setting.[20,21]

With recent rapid advances in molecular techniques, it is likely that massively parallel next generation sequencing will become widely available in coming years.[22] This will provide an opportunity to perform more comprehensive molecular evaluation of a tumour from data generated in a single assay including mutation analysis, copy number changes, structural rearrangements and

mutation burden. Although challenges remain in performing detailed analysis in a timely fashion within the constraints of a diagnostic setting, this will provide an unprecedented opportunity to incorporate molecular data into routine pathological evaluation and provide new insights into diagnosis, and prognostic and predictive biomarkers as well as tumour classification.

While some studies have shown correlation between mutation burden, gene signatures and/or PDL1 expression and response to immunotherapies, at present there are no biomarkers with sufficiently high sensitivity or specificity to be of clinical utility in routine practice.

References

1 Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, Gonzalez R, Robert C, Schadendorf D, Hassel JC, Akerley W, van den Eertwegh AJ, Lutzky J, Lorigan P, Vaubel JM, Linette GP, Hogg D, Ottensmeier CH, Lebbe C, Peschel C, Quirt I, Clark JI, Wolchok JD, Weber JS, Tian J, Yellin MJ, Nichol GM, Hoos A and Urba WJ (2010). Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med* 363(8):711-723.

2 Robert C, Thomas L, Bondarenko I, O'Day S, Weber J, Garbe C, Lebbe C, Baurain JF, Testori A, Grob JJ, Davidson N, Richards J, Maio M, Hauschild A, Miller WH, Jr., Gascon P, Lotem M, Harmankaya K, Ibrahim R, Francis S, Chen TT, Humphrey R, Hoos A and Wolchok JD (2011). Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. *N Engl J Med* 364(26):2517-2526.

3 Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Cowey CL, Lao CD, Schadendorf D, Dummer R, Smylie M, Rutkowski P, Ferrucci PF, Hill A, Wagstaff J, Carlino MS, Haanen JB, Maio M, Marquez-Rodas I, McArthur GA, Ascierto PA, Long GV, Callahan MK, Postow MA, Grossmann K, Sznol M, Dreno B, Bastholt L, Yang A, Rollin LM, Horak C, Hodi FS and Wolchok JD (2015). Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. *N Engl J Med* 373(1):23-34.

4 Robert C, Long GV, Brady B, Dutriaux C, Maio M, Mortier L, Hassel JC, Rutkowski P, McNeil C, Kalinka-Warzocha E, Savage KJ, Hernberg MM, Lebbe C, Charles J, Mihalcioiu C, Chiarion-Sileni V, Mauch C, Cognetti F, Arance A, Schmidt H, Schadendorf D, Gogas H, Lundgren-Eriksson L, Horak C, Sharkey B, Waxman IM, Atkinson V and Ascierto PA (2015). Nivolumab in previously untreated melanoma without BRAF mutation. *N Engl J Med* 372(4):320-330.

5 Robert C, Schachter J, Long GV, Arance A, Grob JJ, Mortier L, Daud A, Carlino MS, McNeil C, Lotem M, Larkin J, Lorigan P, Neyns B, Blank CU, Hamid O, Mateus C, Shapira-Frommer R, Kosh M, Zhou H, Ibrahim N, Ebbinghaus S and Ribas A (2015). Pembrolizumab versus Ipilimumab in Advanced Melanoma. *N Engl J Med* 372(26):2521-2532.

6 Chapman PB, Hauschild A and Robert C et al (2011). Improved survival with vemurafenib in melanoma with BRAF V600E mutation. *N Engl J Med* 364:2507-2516.

7 Flaherty KT, Puzanov I and Kim KB et al (2010). Inhibition of mutated, activated BRAF in metastatic melanoma. *N Engl J Med* 363:809-819.

8 Falchook GS, Long GV, Kurzrock R, Kim KB, Arkenau TH, Brown MP, Hamid O, Infante JR, Millward M, Pavlick AC, O'Day SJ, Blackman SC, Curtis CM, Lebowitz P, Ma B, Ouellet D and Kefford RF (2012). Dabrafenib in patients with melanoma, untreated brain metastases, and other solid tumours: a phase 1 dose-escalation trial. *Lancet* 379(9829):1893-1901.

9 Hauschild A, Grob JJ, Demidov LV, Jouary T, Gutzmer R, Millward M, Rutkowski P, Blank CU, Miller WH, Jr., Kaempgen E, Martin-Algarra S, Karaszewska B, Mauch C, Chiarion-Sileni V, Martin AM, Swann S, Haney P, Mirakhur B, Guckert ME, Goodman V and Chapman PB (2012). Dabrafenib in BRAF-mutated metastatic melanoma: a multicentre, open-label, phase 3 randomised controlled trial. *Lancet* 380(9839):358-365.

10 Long GV, Trefzer U, Davies MA, Kefford RF, Ascierto PA, Chapman PB, Puzanov I, Hauschild A, Robert C, Algazi A, Mortier L, Tawbi H, Wilhelm T, Zimmer L, Switzky J, Swann S, Martin AM, Guckert M, Goodman V, Streit M, Kirkwood JM and Schadendorf D (2012). Dabrafenib in patients with Val600Glu or Val600Lys BRAF-mutant melanoma metastatic to the brain (BREAK-MB): a multicentre, open-label, phase 2 trial. *Lancet Oncol* 13(11):1087-1095.

11 Flaherty KT, Infante JR, Daud A, Gonzalez R, Kefford RF, Sosman J, Hamid O, Schuchter L, Cebon J, Ibrahim N, Kudchadkar R, Burris HA, 3rd, Falchook G, Algazi A, Lewis K, Long GV, Puzanov I, Lebowitz P, Singh A, Little S, Sun P, Allred A, Ouellet D, Kim KB, Patel K and Weber J (2012). Combined BRAF and MEK inhibition in melanoma with BRAF V600 mutations. *N Engl J Med* 367(18):1694-1703.

12 Larkin J, Ascierto PA, Dreno B, Atkinson V, Liszkay G, Maio M, Mandala M, Demidov L, Stroyakovskiy D, Thomas L, de la Cruz-Merino L, Dutriaux C, Garbe C, Sovak MA, Chang I, Choong N, Hack SP, McArthur GA and Ribas A (2014). Combined vemurafenib and cobimetinib in BRAF-mutated melanoma. *N Engl J Med* 371(20):1867-1876.

13 Long GV, Stroyakovskiy D, Gogas H, Levchenko E, de Braud F, Larkin J, Garbe C, Jouary T, Hauschild A, Grob JJ, Chiarion Sileni V, Lebbe C, Mandala M, Millward M, Arance A, Bondarenko I, Haanen JB, Hansson J, Utikal J, Ferraresi V, Kovalenko N, Mohr P, Probachai V, Schadendorf D, Nathan P, Robert C, Ribas A, DeMarini DJ, Irani JG, Casey M, Ouellet D, Martin AM, Le N, Patel K and Flaherty K (2014). Combined BRAF and MEK inhibition versus BRAF inhibition alone in melanoma. *N Engl J Med* 371(20):1877-1888.

14 Robert C, Karaszewska B, Schachter J, Rutkowski P, Mackiewicz A, Stroiakovski D, Lichinitser M, Dummer R, Grange F, Mortier L, Chiarion-Sileni V, Drucis K, Krajsova I, Hauschild A, Lorigan P, Wolter P, Long GV, Flaherty K, Nathan P, Ribas A, Martin AM, Sun P, Crist W, Legos J, Rubin SD, Little SM and Schadendorf D (2015). Improved overall survival in melanoma with combined dabrafenib and trametinib. *N Engl J Med* 372(1):30-39.

15 Long GV, Stroyakovskiy D, Gogas H, Levchenko E, de Braud F, Larkin J, Garbe C, Jouary T, Hauschild A, Grob JJ, Chiarion-Sileni V, Lebbe C, Mandala M, Millward M, Arance A, Bondarenko I, Haanen JB, Hansson J, Utikal J, Ferraresi V, Kovalenko N, Mohr P, Probachai V, Schadendorf D, Nathan P, Robert C, Ribas A, DeMarini DJ, Irani JG, Swann S, Legos JJ, Jin F, Mookerjee B and Flaherty K (2015). Dabrafenib and trametinib versus dabrafenib and placebo for Val600 BRAF-mutant melanoma: a multicentre, double-blind, phase 3 randomised controlled trial. *Lancet* 386(9992):444-451.

16 Long GV, Menzies AM, Nagrial AM, Haydu LE, Hamilton AL, Mann GJ, Hughes TM, Thompson JF, Scolyer RA and Kefford RF (2011). Prognostic and clinicopathologic associations of oncogenic BRAF in metastatic melanoma. *J Clin Oncol* 29(10):1239-1246.

17 Jakob JA, Bassett RL, Jr., Ng CS, Curry JL, Joseph RW, Alvarado GC, Rohlfs ML, Richard J, Gershenwald JE, Kim KB, Lazar AJ, Hwu P and Davies MA (2012). NRAS mutation status is an independent prognostic factor in metastatic melanoma. *Cancer* 118(16):4014-4023.

18 Gerami P, Jewell SS and Morrison LE et al (2009). Fluorescence in situ hybridization (FISH) as an ancillary diagnostic tool in the diagnosis of melanoma. *Am J Surg Pathol* 33:1146-1156.

19 Bastian BC, Wesselmann U, Pinkel D and Leboit PE (1999). Molecular cytogenetic analysis of Spitz nevi shows clear differences to melanoma. *J Invest Dermatol* 113(6):1065-1069.

20 Ko JS, Clarke LE, Minca EC, Brown K, Flake DD, 2nd and Billings SD (2018). Correlation of melanoma gene expression score with clinical outcomes on a series of melanocytic lesions. *Hum Pathol*.

21 Ko JS, Matharoo-Ball B, Billings SD, Thomson BJ, Tang JY, Sarin KY, Cai E, Kim J, Rock C, Kimbrell HZ, Flake DD, 2nd, Warf MB, Nelson J, Davis T, Miller C, Rushton K, Hartman AR, Wenstrup RJ and Clarke LE (2017). Diagnostic Distinction of Malignant Melanoma and Benign Nevi by a Gene Expression Signature and Correlation to Clinical Outcomes. *Cancer Epidemiol Biomarkers Prev* 26(7):1107-1113.

22 Bauer J (2017). The Molecular Revolution in Cutaneous Biology: Era of Cytogenetics and Copy Number Analysis. *J Invest Dermatol* 137(5):e57-e59.