Cancer Research

Clinical Research 2: Novel Therapeutics and Biomarkers of Response

Evaluation and development of an immunohistochemical procedure to monitor phospho-S6 as a biomarker for the activity of RAD001.

Volume 65, Issue 9 Supplement, pp. 109-110
American Association for Cancer Research

AUTHOR INFORMATION

1. **Michael Stumm,**
2. **Terence M. O'Reilly,**
3. **Sabine Zumstein-Mecker,**
4. **Caroline Fux,**
5. **Maya Walker, and**
6. **Heidi A. Lane**

1. *Novartis Institutes for Biomedical Research, Basel, Switzerland*
Proc Amer Assoc Cancer Res, Volume 46, 2005

Abstract

469
RAD001 is an orally active mTOR pathway inhibitor in clinical evaluation. The mTOR kinase regulates both S6 kinase (S6K) (a translational activator) and 4E-BP1 (a translational inhibitor), so that mTOR inhibition will disrupt the regulation of protein synthesis. In experimental and early clinical studies, RAD001 has demonstrated the inhibition of S6K-1 inhibition occurs in peripheral blood lymphocytes (PBL) and in various tissues, including tumors, and is well-aligned with the pharmacokinetics of RAD001. This suggests that S6K activity is a valuable marker for the activity of RAD001. As immunohistochemical (IHC) assays are broadly applicable, we sought to prepare a phospho-S6 protein (pS6) IHC assay for clinical use. A549 cell pellets or cytospin preparations from cells, with or without RAD001 exposure, demonstrated "on-off" staining with anti-pS6 antibodies which recognize distinct phosphorylated-epitopes (Ser 235/236 or Ser 240/246), reflecting the activated S6K pathway. Specificity of staining was confirmed by immunoblotting. CA20948 tumors from rats receiving an effective RAD001 treatment demonstrated the absence of pS6 staining as compared with vehicle-treatment (both Ser 235/236 or Ser 240/246). In addition, skin samples from

these rats showed marked reduction in pS6 staining. Phosphospecificity of the Ser 240/246 mAb was demonstrated by calf intestinal phosphatase (CIP) pretreatment of tissue sections yielding no staining. Furthermore storage of tissue sections at room temperature for 2 months markedly reduced immunoreactivity. IHC performed with A549 cells and patient-derived tumor material with different lots of Ser 240/246 indicated lot-specific intensity of immunoreactivity. Tissue microarrays of rat and mouse organs demonstrated pS6 detected by Ser 240/246 in various organs, notably the hair follicles where a response to RAD001 was clearly evident by IHC. Lastly a series of human breast and colon cancer samples showed the utility of IHC in detecting pS6. Hence, the development of the IHC method for following S6 phosphorylation changes in tumors and surrogate tissues provides a useful marker of RAD001 activity during clinical studies.

- American Association for Cancer Research