INTRODUCTION
The importance of hypoxia as a potential obstacle to the successful treatment of head and neck squamous cell carcinoma (HNSCC) has been recognized for a long time. The presence of hypoxia as detected by oxygen electrodes has been shown to be an adverse prognostic factor in head and neck cancer treated with radiotherapy (RT) (1). Progress has been hampered by the difficulties in measuring hypoxia in patients with HNSCC enrolled in clinical trials and by the limited efficacy observed with interventions designed to overcome hypoxia in HNSCC.

Treatment approaches to overcome hypoxia have included the use of hypoxic cell sensitizers, hyperbaric oxygen, high linear energy transfer radiation, and accelerated RT with carbogen and nicotinamide. A conceptually different approach is the incorporation of bioreductive agents, such as the benzotriazine compound, tirapazamine (3-amino-1,2,4-benzotriazine 1,4-dioxide) (TPZ). TPZ is a hypoxic cell cytotoxin, rather than a hypoxic cell sensitizer, which maintains its differential toxicity relative to aerobic cells over a wide range of low oxygen concentrations (2).

In the 1990s, the focus of TPZ development was on testing this drug in combination with platinum-based chemotherapy, predominantly for metastatic non–small-cell lung cancer. Several small studies have combined TPZ with RT, although often for diseases for which the efficacy of RT is limited and the importance of hypoxia as a mechanism limiting efficacy has not been established. One previous trial of TPZ and RT in HNSCC has been published (3). Because the evidence of the importance of hypoxia in limiting efficacy in local-regionally advanced HNSCC treated with RT is strong, we proposed that locally advanced HNSCC would be an excellent model to determine the benefit of adding a hypoxic cytotoxin to RT. Furthermore, because cisplatin-based chemoradiotherapy was emerging as superior to RT alone and impressive synergy was present between cisplatin and TPZ in preclinical models, it made sense to combine both drugs with RT. The phase I trial established an acceptable schedule and doses and provided promising efficacy data (4). This led to the randomized phase II trial (Trans-Tasman Radiation Oncology Group [TROG] 98.02) conducted by the TROG, which established the feasibility of the regimen in a multi-institutional setting (5). Significant, but manageable, acute toxicity was observed, and the promising efficacy of this regimen was confirmed.

The results of these trials led to this regimen being tested in two large international phase III trials. In both trials, the control arm receive cisplatin 100 mg/m² during Weeks 1, 4, and 7 of RT, and the experimental arm received cisplatin, TPZ, and RT at the same doses and schedule used in TROG 98.02. The first trial, known as the Headstart trial, was a collaboration between TROG and Sanofi-Aventis. A total of 861 patients were accrued during 29 months from 88 centers in 13 countries. Accrual was completed in April 2005, and the final results will be available in late 2007. The second trial, known as TRACE, ceased accrual early because an excess of early deaths in the TPZ arm. This was a most unexpected finding, because no difference had been found in the incidence of early deaths or major toxicity between the two arms in the Headstart trial.

No reference standard is available for the measurement of hypoxia in human tumors, although polarographic oxygen probe measurements (Eppendorf microelectrode) have been...
most widely studied and have been reported to show a correlation between hypoxia and adverse outcomes with RT \( (1) \); however, this technology has a number of significant limitations that precludes its use in multi-institutional trials or clinical practice. Functional imaging of hypoxia might overcome some of these problems, because it is less invasive, can be repeated during treatment, will distinguish viable hypoxic tumor from nonviable necrotic areas, and could potentially detect all hypoxic areas below a certain threshold. Preliminary studies with \([18F]\)-misonidazole positron emission tomography (PET) (FMISO) have demonstrated that hypoxia can be detected in human cancers using functional imaging \( (6) \). We incorporated hypoxic imaging into the TPZ trials from the outset and sought to answer a number of questions. Hypoxic imaging with FMISO has been conducted at the Peter MacCallum Cancer Centre on 74 patients enrolled on the phase I and II tirapazamine trials; 16 patients on the phase I trial, 45 patients enrolled on the randomized phase II trial, and 13 patients enrolled on the extension phase of the phase II. The 13 patients on the extension phase had both FMISO and \([18F]\)-azomycin arabinoside (FAZA) scans. In addition, 47 patients in the Headstart Phase III trial underwent FAZA imaging studies.

**INCIDENCE OF HYPOXIA IN HNSCC AS DETECTED BY PET**

Hypoxia was detected in 58 (78%) of 74 patients in the primary tumor and/or within the lymph nodes. Patients enrolled in the phase I trial and the extension phase of the phase II trial had more advanced disease than did patients enrolled in the randomized phase II trial. Of the 16, 13, and 45 patients enrolled in the phase I study, the extension phase study, and the randomized trial, 15, 13, and 33 had Stage 4 disease. The corresponding incidence of hypoxia was 81% (13 of 16), 100% (13 of 13), and 71% (32 of 45). Hypoxia in the primary tumor was related to a more-advanced T stage \( (p < 0.001) \). Nodal hypoxia was related to a more advanced nodal stage \( (p < 0.001) \).

**HYPOXIA DETECTED BY HYPOXIC PET IMAGING AN ADVERSE PROGNOSTIC FACTOR**

Of the 10 patients who received the chemoboost regimen in the randomized phase II trial, 1 with no PET evidence of hypoxia in the primary tumor or nodes developed locoregional failure (LRF) compared with 8 of 13 of those with hypoxia. Also, the interval to LRF was significantly shorter in the hypoxic patients \( (\text{exact log–rank } p = 0.035; \text{hazard ratio } [HR], 7.8) \) \( (7) \).

**PRESENCE OF HYPOXIA ON FUNCTIONAL IMAGING PREDICTS BENEFIT FROM ADDITION OF TPZ**

Although 8 of 13 patients with hypoxia treated with the chemoboost developed LRF, only 1 of 19 patients with hypoxic tumors treated with the TPZ-containing regimen \( (\text{cisplatin plus TPZ}) \) developed LRF. The interval to LRF was significantly shorter in the chemoboost patients \( (p = 0.001; \text{HR}, 17) \). The results at the primary site alone were also quite striking. For patients with hypoxic primary tumors treated with cisplatin and TPZ, 0 of 8 had local failure compared with 6 of 9 treated with the chemoboost \( (p = 0.011) \). The importance of hypoxia in the primary tumor was further supported by the preliminary results from the FAZA imaging studies in the Phase III trial, with 0 of 18 hypoxic primary tumors treated with cisplatin and TPZ failing locally compared with 2 of 9 treated with cisplatin. These results suggest that hypoxic PET imaging has the potential to identify the patients most likely to benefit from treatment with this TPZ-containing chemoradiotherapy regimen.

**CORRELATION BETWEEN PRESENCE OF HYPOXIA IN PRIMARY SITE AND PRESENCE OF HYPOXIA IN LYMPH NODES**

Hypoxia was detected in 85% of the node-positive patients (56 of 66) in the primary tumor and/or the lymph nodes. No evidence was found that hypoxia in the primary tumor was related to an increased likelihood of hypoxia in the lymph nodes. In node-positive patients, 17 (63%) of 27 patients with nonhypoxic primary tumors had hypoxia in the lymph nodes compared with 19 (49%) of 39 with hypoxic primary tumors. In only 29 (44%) of 66 patients was concordance present between the hypoxia results in the primary and nodes. This lack of correlation between hypoxia in the primary and lymph nodes could have significant implications for hypoxia detection tests using invasive sampling from one site.

**ARE SOME HEAD-AND-NECK PRIMARY SITES MORE LIKELY TO BE HYPOXIC?**

Hypoxia was found in the primary and/or lymph nodes in 2 (40%) of 5 oral cavity, 41 (80%) of 51 oropharyngeal, 10 (91%) of 11 hypopharyngeal, and 5 (71%) of 7 laryngeal primary tumors. It was detected in all subsites of oropharyngeal primary tumors apart from those arising in the soft palate.

**ASSOCIATION BETWEEN HYPOXIA ON PET AND RISK OF DEVELOPING DISTANT METASTASES**

Of the 16 patients without hypoxia in the primary tumor and/or nodes, 1 had treatment failure at distant sites as part of the first failure compared with 13 of 58 with hypoxia \( (HR, 3.2; p = 0.33) \). The only variable that was significantly associated with distant failure was a hypopharyngeal primary site \( (HR, 3.92; p = 0.021) \).

**DOES FAZA HAVE ADVANTAGES COMPARED WITH FMISO?**

We have also conducted intrapatient comparison imaging studies of \(^{18}F\)-MISO and \(^{18}F\)-AZA PET and found a similar distribution of tumor hypoxia for both tracers; however, less background tracer accumulation occurred with FAZA, making it a better marker for imaging hypoxia in this patient group.
CONCLUSION

Hypoxic PET imaging is a promising method of identifying patients with hypoxic tumors. In trials that select patients with very advanced disease, the proportion of patients with hypoxia detectable in the primary and/or nodes may be very high. However, trials that are open to most patients with Stage 3 and 4 disease might have a significant proportion without hypoxia. The benefit from interventions to overcome hypoxia could be missed in trials of unselected HNSCC patients and might only be evident by targeting the subgroup with tumor hypoxia. In addition, hypoxic PET imaging can provide information that is not easily obtainable with hypoxia assays that require invasive intervention (e.g., hypoxia status in both primary tumor and lymph nodes). Our results suggest that hypoxia in the primary might be more important than nodal hypoxia in determining the risk of LRF and predicting the benefit from the addition of a hypoxic cytotoxin. The increasing availability of PET scanners makes it feasible to include hypoxic imaging in future trials of hypoxia targeting therapy.

REFERENCES