Effects of lapatinib monotherapy: results of a randomised phase II study in therapy-naive patients with locally advanced squamous cell carcinoma of the head and neck

BACKGROUND: Lapatinib is a dual inhibitor of epidermal growth factor receptor (EGFR) and human EGFR-2 (HER-2) tyrosine kinases. This study investigated the pharmacodynamic and clinical effects of lapatinib in patients with locally advanced squamous cell carcinoma of the head and neck (SCCHN).

METHODS: In total, 107 therapy-naive patients with locally advanced SCCHN were randomised (2:1) to receive lapatinib or placebo for 2–6 weeks before chemoradiation therapy (CRT). Endpoints included apoptosis and proliferation rates, clinical response, and toxicity.

RESULTS: Versus placebo, lapatinib monotherapy did not significantly increase apoptosis detected by terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-biotin nick-end labelling or caspase-3 assays. A statistically significant decrease in proliferation using Ki67 assay was observed (P = 0.030). In a subset of 40 patients that received ≥4 weeks of lapatinib or placebo, objective response rate (ORR) was 17% (n = 4/24) vs 0% (n = 0/16). In the lapatinib single-agent responders, all had EGFR overexpression, 50% had EGFR amplification, and 50% had HER2 expression by immunohistochemistry (including one patient with HER2 amplification). However, these patients showed variable modulation of apoptosis, proliferation, and phosphorylated EGFR on drug treatment. Following CRT, there was a statistically non-significant difference in ORR between lapatinib (70%) and placebo (53%). There was no clear correlation between changes in apoptosis or proliferation and response to chemoradiation. Mucosal inflammation, asthenia, odynophagia, and dysphagia were the most commonly reported adverse events with lapatinib.

CONCLUSION: Short-term lapatinib monotherapy did not demonstrate apoptotic changes, but provided evidence of clinical activity in locally advanced SCCHN, and warrants further investigation in this disease.

Keywords: epidermal growth factor receptor; lapatinib; squamous cell carcinoma

The epidermal growth factor receptor (EGFR) is involved in normal cell growth and differentiation. EGFR is particularly important in the pathogenesis of squamous cell carcinoma of the head and neck (SCCHN) (Kalyankrishna and Grandis, 2006), with reported overexpression in approximately 90% of tumours (Grandis and Teweary, 1993). EGFR promotes growth and survival through several oncogenic signalling pathways, and its overexpression in SCCHN correlates with poor prognosis, short disease-free survival, and increased locoregional recurrence (Ang et al, 2002; Eriksen et al, 2004; Hitt et al, 2005; Chung et al, 2006), and makes it an attractive therapeutic target for therapy (Bonner et al, 2002; Johns et al, 2003; Harari et al, 2007).

Lapatinib is a reversible dual inhibitor of both EGFR and human EGFR-2 (HER2) tyrosine kinases, which in turn inhibits activation of downstream signalling pathways such as Erk1/2 and Akt in cell lines and xenografts (Xia et al, 2002; Rusnak et al, 2007). Lapatinib elicits cytostatic or cytotoxic anti-tumour effects, depending on the cell type (Chu et al, 2005; Coley et al, 2006; Rusnak et al, 2007; Konecny et al, 2008), and has demonstrated clinical activity in several solid tumours (Spector et al, 2005; Burstein et al, 2008; Cameron et al, 2008; Ravaud et al, 2008).
Lapatinib has shown a single-agent activity in in vitro and in vivo xenograft studies in human head and neck cancer cell lines (GSK, data on file). In addition, lapatinib has been safely combined with chemoradiation in phase I study, with a recommended dose of 1500 mg day\(^{-1}\) for future trials (Harrington et al., 2009). A randomised-phase II study of chemoradiation plus lapatinib, followed by maintenance lapatinib versus chemoradiation plus placebo, followed by placebo, has shown a statistically non-significant 17%-point superiority in favour of lapatinib-treated patients for complete and overall response at 6 months post-chemoradiation (Harrington et al., 2010). Lapatinib is currently in phase III trial with chemoradiation in patients with high-risk features after surgical treatment of stage III/IVA head and neck cancer.

Although the molecular effects of many targeted-anticancer agents are often characterised in vitro, correlation of such effects with clinical outcome has only started to be adopted in proof-of-concept trials (Goulart et al., 2007; Banerji et al., 2008). Such an approach often uses apoptosis or proliferation endpoints (Sarker and Workman, 2007; Doroshaw and Pachment, 2008). Spector et al. (2005) have recently shown that the 3-week treatment with lapatinib in patients with advanced malignancies resulted in increased tumour cell apoptosis, occurring in patients with evidence of tumour regression. The objectives of this study were to explore the biological effects of lapatinib on apoptosis and proliferation in pre- and post-treatment tumour tissues in patients with locally advanced SCCHN.

**MATERIALS AND METHODS**

This was a multinational, randomised, single-blinded, placebo-controlled study, conducted at 10 centres in six countries. The study was approved by independent ethics committees and regulatory agencies, and was carried out in accordance with the Declaration of Helsinki and good clinical practice. All patients gave written informed consent before enrolment.

Adults of at least 18 years of age with newly diagnosed stage III/IVA/IVB SCCHN undergoing chemoradiation therapy (CRT) were eligible. Other criteria included Eastern Cooperative Oncology Group performance status of 0, 1, or 2; adequate renal, hepatic, and bone marrow function; and normal left ventricular ejection fraction assessed by echocardiogram or multigated acquisition scan. Exclusion criteria included evidence of distant metastasis (stage IVC), earlier systemic chemotherapy, radiotherapy, or required concomitant use of cytochrome P450 3A4 inducers or inhibitors.

Figure 1A shows the study design. Patients were randomised 2:1 to receive lapatinib (1500 mg day\(^{-1}\)) or placebo, and stratified by tumour site and performance status. Treatment with lapatinib/placebo continued for 2–6 weeks until the start of CRT. The 'window of opportunity' represented the period of time required for radiotherapy preparation. The initiation of CRT was not delayed in patients receiving either lapatinib or placebo, as the monotherapy phase lasted no longer than local standards allowed. The mean time between commencing lapatinib/placebo and initiating CRT was 25.8 days for both arms. Chemotherapy schedule and CRT was mandated as concomitant cisplatin and radiation, and followed the local standard. Conventional radiotherapy was given to a total of 66–70 Gy given over 6–7 weeks.

Medications that inhibit or induce cytochrome P450 3A4 were prohibited. Lapatinib could be withheld for up to 1 week for any grade 3 or 4 toxicity, and permanently discontinued if grade 3 or 4 interstitial pneumonitis or cardiac dysfunction occurred.

**Study assessments**

Baseline assessments included demography, medical history, physical examination, performance status, panendoscopy, echocardiogram, multigated acquisition scan, haematology, and clinical chemistry. Clinical examination and laboratory tests were repeated during treatment and at follow-up. Adverse events and serious adverse events were collected throughout the study and were graded using the National Cancer Institute’s Common Terminology Criteria for Adverse Events, v 3.0 (Cancer Therapy Evaluation Program, 2006).

Objective response rate (ORR) was assessed by performing radiologic examination at baseline, 8 and 12 weeks post-CRT. Additional scans were performed before the commencement of CRT for patients receiving at least 4 weeks of lapatinib/placebo. Efficacy was defined according to Response Evaluation Criteria in Solid Tumours criteria, version 1.0. (Therasse et al., 2000) All scans and clinical data were reviewed centrally by an independent review board (BioClinica, Newtown, PA, USA), and all readers were blinded to treatment. Follow-up beyond 3 months post-CRT was not part of the protocol.

A positron emission tomography (PET) substudy was conducted at participating centres. Thirty-five subjects who participated in the trial agreed to the substudy with fluorodeoxyglucose (FDG)–PET imaging at baseline and the follow-up time point. The sites that were qualified to conduct FDG–PET scans adhered to guidelines regarding the scans. The first scan of the head and neck was performed at screening, before biopsy, to obviate effects of the biopsy procedure on glucose uptake. The second scan was conducted at the end of the week-2 lapatinib treatment (again, before biopsy). The procedure further included: (1) administration of approximately 300–370 MBq FDG; (2) 60 minutes ± 10 minutes of rest; (3) an attenuation scan for transmission correction; (4) whole-body emission scan; and (5) whole-body postcontrast computed tomography scan.

Analysis included acquisition of both quantitative measurements of standard uptake values (SUVs), as well as a qualitative assessment. The target and non-target lesions were analysed, based on SUV levels by a PET nuclear medicine expert. At baseline and post-monotherapy time points, approximately 300–370 MBq FDG was administered. Acquisition of SUV occurred after 60 minutes (± 10 minutes) of rest.

**Tissue acquisition**

Fresh tumour biopsies were obtained at time of study enrolment (day 0) and after 2 weeks of study participation (day 14). Biopsies were immediately fixed in 10% neutral-buffered formalin containing phosphatase inhibitors before paraffin-embedded sections were prepared. Hematoxylin and eosin staining was used to confirm the presence of tumour.

**Biological evaluations**

Aptosis was measured using immunohistochemistry (IHC) in paired tumour samples by both terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labelling (TUNEL) and active caspase-3 assays. The expression patterns of Ki-67, EGFR, HER2, phosphorylated-EGFR (pEGFR), p16, and p53 were also studied by IHC (details of antibody tests can be found in Supplementary Table 1). HER2 and EGFR gene amplification were also studied by IHC (details of antibody tests can be found in Supplementary Table 1). HER2 and EGFR gene amplification were assessed using fluorescence in situ hybridisation.

**Statistical analysis**

The primary endpoint was apoptotic index (AI), which was calculated at baseline and after treatment (percent stained nuclei/total nuclei). Secondary endpoints included proliferation rate given by proliferation index (PI) (percent proliferating cells/total number of cells), ORR, adverse events, and correlative biomarker analyses.
Using a two-sided significance level of 5 and 80% power, 90 patients were required to detect a 24% difference in apoptosis between the two groups. To allow for dropouts during tissue acquisition, a total of 107 patients were randomised.

The change from baseline in AI was analysed using an analysis of covariance model, adjusted for the baseline strata. All other endpoints were summarised as appropriate.

All clinical and biological analyses presented were based on the intent-to-treat (ITT) population, which comprised all patients randomised to study treatment. The evaluable population for radiologic assessment was defined as all patients who completed CRT and had baseline and follow-up scans. This population was used for a more accurate estimation of clinical benefit.

**RESULTS**

**Patient characteristics**

Between March 2006 and July 2007, a total of 107 patients were randomised to receive lapatinib (n = 71) or placebo (n = 36). The
Table 1  Baseline patient characteristics (ITT population)  

| Characteristic                  | Lapatinib (n = 71) | Placebo (n = 36) |
|---------------------------------|--------------------|-----------------|
| Age, years                      |                    |                 |
| Median (range)                  | 58 (33–80)         | 55 (37–78)      |
| Sex, n (%)                      |                    |                 |
| Female                          | 16 (23)            | 4 (11)          |
| Male                            | 55 (77)            | 32 (89)         |
| ECOG performance status, n (%)  |                    |                 |
| 0–1                             | 70 (99)            | 35 (97)         |
| 2                               | 1 (1)              | 1 (3)           |
| Primary tumour site, n (%)      |                    |                 |
| Oral cavity                     | 27 (38)            | 7 (19)          |
| Oropharynx                      | 24 (34)            | 16 (44)         |
| Larynx                          | 12 (17)            | 6 (17)          |
| Hypopharynx                     | 8 (11)             | 7 (19)          |
| Histologic grade at initial diagnosis, n (%) |                |                 |
| Well differentiated             | 24 (34)            | 11 (31)         |
| Moderately differentiated       | 21 (30)            | 11 (31)         |
| Poorly differentiated           | 8 (11)             | 4 (11)          |
| Can not be assessed             | 17 (24)            | 9 (25)          |
| Missing                         | 1 (1)              | 1 (3)           |
| T-category, n (%)               |                    |                 |
| T1                              | 1 (1)              | 0               |
| T2                              | 10 (14)            | 6 (17)          |
| T3                              | 26 (37)            | 10 (28)         |
| T4                              | 34 (48)            | 20 (56)         |
| N-category, n (%)               |                    |                 |
| N0                              | 19 (27)            | 3 (8)           |
| N1                              | 12 (17)            | 8 (22)          |
| N2                              | 39 (55)            | 23 (64)         |
| N3                              | 1 (1)              | 2 (6)           |
| TNM staging, n (%)              |                    |                 |
| III                             | 20 (28)            | 7 (19)          |
| IV                              | 51 (72)            | 29 (81)         |
| p16 expression by IHC, n (%)    |                    |                 |
| n                               | 66                 | 30              |
| 0, 1+                           | 34 (52)            | 10 (33)         |
| 2+, 3+                          | 23 (35)            | 18 (60)         |
| Missing                         | 9 (14)             | 2 (7)           |
| EGFR protein expression by IHC, n (%) |             |                 |
| n                               | 69                 | 36              |
| 0, 1+                           | 6 (6)              | 4 (11)          |
| 2+, 3+                          | 64 (93)            | 30 (83)         |
| Missing                         | 1 (1)              | 2 (6)           |
| pEGFR expression by IHC, n (%)  |                    |                 |
| n                               | 69                 | 36              |
| 0, 1+                           | 40 (58)            | 19 (53)         |
| 2+, 3+                          | 28 (41)            | 15 (42)         |
| Missing                         | 1 (1)              | 2 (6)           |
| EGFR gene amplification by FISH, n (%) |            |                 |
| n                               | 67                 | 33              |
| Amplified                       | 19 (28)            | 13 (39)         |
| Oral cavity                     | 3/25 (12)          | 2/7 (29)        |
| Oropharynx                      | 9/23 (39)          | 7/15 (47)       |
| Larynx                          | 2/11 (18)          | 1/6 (17)        |
| Hypopharynx                     | 5/8 (63)           | 3/5 (60)        |
| Not amplified                   | 48 (72)            | 20 (61)         |
| Oral cavity                     | 22/25 (88)         | 5/7 (71)        |
| Oropharynx                      | 14/23 (61)         | 8/15 (53)       |
| Larynx                          | 9/11 (82)          | 5/6 (83)        |
| Hypopharynx                     | 3/8 (38)           | 2/5 (40)        |

Abbreviations: ECOG = Eastern Cooperative Oncology Group; EGFR = epidermal growth factor receptor; FISH = fluorescent in situ hybridisation; HER2 = human EGFR receptor-2; IHC = immunohistochemistry; pEGFR = phosphorylated EGFR; TNM = tumour, node, metastasis.

study reached the last patient’s last visit in December 2007. Figure 1B shows the flow of patients through the trial and highlights the specific patient populations that will be discussed throughout the manuscript: the ITT population comprised 107 patients randomised to either lapatinib or placebo; the modified ITT population included 84 patients in whom pre- and post-treatment biopsies were obtained for analysis of biological endpoints; the evaluable population was made up of 88 patients who had CT/MRI scans at 12 weeks post-chemoradiation, and who were evaluable for treatment response; the monotherapy efficacy population comprised a subgroup of 40 patients who underwent CT/MRI scanning after receiving at least 4 weeks of lapatinib/placebo before chemoradiation. A total of 84 and 88 patients were considered evaluable for apoptosis/proliferation and post-CRT clinical activity, respectively. Table 1 shows the baseline demographics. The two treatment groups were generally well balanced. The incidence of p16-positive tumours (IHC 2+/3+) was 43% (35% for the lapatinib group and 60% for the placebo group). EGFR overexpression (IHC 2+/3+) was seen in 93 and 83% of lapatinib and placebo patients, respectively; EGFR gene amplification was seen in 28 and 39% of patients. EGFR amplification was highest in hypopharyngeal tumours (n = 8/13 (62%)), and lowest in oral cavity (16%) and laryngeal tumours (17%). HER2 overexpression and gene amplification accounted for only 7% and 4% in total, respectively.

Eighty-two percent of lapatinib and 92% of placebo patients were at least 80% compliant. The planned radiotherapy dose was given to 88 and 81% of lapatinib and placebo patients, respectively. For the lapatinib and placebo groups, the median radiation doses given to 88 and 81% of lapatinib and placebo patients, respectively. EGFR overexpression (IHC 2+/3+) was observed in 28 and 39% of patients. EGFR gene amplification was observed in 28 and 31% of patients. EGFR amplification was highest in hypopharyngeal tumours (n = 8/13 (62%)), and lowest in oral cavity (16%) and laryngeal tumours (17%). HER2 overexpression and gene amplification accounted for only 7% and 4% in total, respectively.

Biological effects of lapatinib

The activation of EGFR (pEGFR, IHC 2+/3+) was observed in 28 (41%) and 15 (42%) patients in the lapatinib and placebo arms (Table 1), of which 24 and 15 patients, respectively, had
Apoptosis induction
Apoptotic cells detected by TUNEL were frequent with lapatinib and placebo, both pre- and posttreatment (Figure 2A–D). Both groups showed similar rates of pretreatment apoptosis, with an AI mean of 3.8 (s.d. = 3.38) and 3.2% (s.d. = 3.14) for lapatinib and placebo, respectively. The posttreatment mean AI was 8.0% (s.d. = 6.66) with lapatinib and 9.4% (s.d. = 12.22) with placebo. The change in AI from pretreatment was not statistically significant for lapatinib versus placebo at the two-sided 5% significance level (difference: -5.4%, s.e. = 2.47, P = 0.030, 95% confidence interval: -10.36% to -0.53%; Figure 3).

Clinical outcome
Forty patients were assessed for response after the monotherapy phase and before CRT. The independently assessed ORR was 17 (n = 4/24) versus 0% (n = 0/16) in the ITT and evaluable populations, respectively, following approximately 4 weeks of lapatinib/placebo treatment. No progressive disease was observed with lapatinib, compared with 25% with placebo (see Table 2 and Figure 4A). From the lapatinib responders, all patients had EGFR overexpression; 50% had EGFR amplification; 50% HER2 expression by IHC, in one of these cases, HER2 gene was amplified; and variable modulation of apoptosis, proliferation, and pEGFR (details in Supplementary Table 2). Two of the responders were positive for p16.

The independently assessed ORR following CRT in the evaluable population was 86 and 63% in the lapatinib and placebo arms, respectively, in the ITT, the response rate was 70 and 53%. A higher number of patients presented with progressive disease in the placebo arm (25%) than in the lapatinib arm (6%). Oropharyngeal and oral cavity tumours were characterised by highest response rate (95% and 91%, respectively) in the lapatinib arm, compared with larynx (70%), hypopharyngeal tumours (67%), or placebo arm (67%, 43%, 60%, and 67%, respectively; see Figure 4B).

In the PET substudy (n = 35), maximum SUV (SUV\text{max}) was reduced from baseline values in 75% of patients treated with lapatinib compared with 36% treated with placebo (see Figure 4C). The change in median SUV\text{max} was -12.9 for lapatinib and +6.1
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for two patients (Table 4).

serious adverse event (6% and 4%, respectively; see Supplementary group (19%), with mucosal inflammation, the most common higher in the placebo/CRT group (36%) than in the lapatinib/CRT placebo (41% grade 3; no grade 4 events).

lapatinib/CRT arm (46% and 4%, respectively), compared with grouping of associated adverse events) were also higher in the group (see Supplementary Table 3). Grade 3 and 4 mucositis (a inflammation (70%) compared with 67% in the placebo/CRT placebo/CRT group, and a slightly higher incidence of mucosal higher incidence of diarrhoea (26%) compared with 6% in the complete duration of the study, the lapatinib/CRT group showed a reported rash (23%) and diarrhoea (22%), which were mainly grades 1 and 2 (see Supplementary Figure 5). Throughout the complete monotherapy phase, only patients receiving lapatinib reported rash (23%) and diarrhoea (22%), which were mainly assessed, with a median −10.9 for lapatinib and +3.2 for placebo.

Safety

In the monotherapy phase, only patients receiving lapatinib reported rash (23%) and diarrhoea (22%), which were mainly grades 1 and 2 (see Supplementary Figure 5). Throughout the complete duration of the study, the lapatinib/CRT group showed a higher incidence of diarrhoea (26%) compared with 6% in the placebo/CRT group, and a slightly higher incidence of mucosal inflammation (70%) compared with 67% in the placebo/CRT group (see Supplementary Table 3). Grade 3 and 4 mucositis (a grouping of associated adverse events) were also higher in the lapatinib/CRT arm (46% and 4%, respectively), compared with placebo (41% grade 3; no grade 4 events).

The reported serious adverse events during or after CRT were higher in the placebo/CRT group (36%) than in the lapatinib/CRT group (19%), with mucosal inflammation, the most common serious adverse event (6% and 4%, respectively; see Supplementary Table 4).

Seven patients experienced cardiac-related events: 2 (3%) in the lapatinib arm and 5 (14%) in the placebo arm. The event was fatal for two patients (n = 1 in each arm).

Twelve deaths were reported (10 and 14% of patients in the lapatinib and placebo arms, respectively). The primary causes were disease under study (6% lapatinib and 8% placebo), ventricular fibrillation (n = 1, placebo), septicaemia (3%, placebo), and intestinal perforation, cardiac arrest, and sudden death (3% each, lapatinib). None of these fatal events was attributed to the study medication.

DISCUSSION

This study set out to investigate whether the biological effects of lapatinib on cell survival and growth pathways predict clinical outcome and to identify any subgroup of patients that may benefit from lapatinib treatment. It was previously shown that activated EGFR modulates the proapoptotic and antiapoptotic pathways (Modjtahedi et al, 1998; Grandis et al, 2000; Ginsberg, 2007; Goel et al, 2007). Therefore, the primary objective assessed the effect of lapatinib on apoptosis. Apoptosis induction was not statistically different compared with placebo, and therefore, the primary endpoint was not met. Indeed, the AI by TUNEL staining increased, following both lapatinib and placebo treatment. Although the possibility that these data may have been affected by artifact introduced during post-treatment biopsy handling should be considered; we believe that the randomised nature of the study should have provided insurance against this risk. The lack of a clear apoptotic signal may be somewhat surprising, as lapatinib is a potent inhibitor of pEGFR and pHER2 in cell-free systems (Rusnak et al, 2001), and induces apoptosis in vitro and in vivo models (Xia et al, 2002; Zhou et al, 2006) as well as clinical studies.
However, similar values have previously been reported for spontaneous apoptosis and apoptosis induction (Hotz et al., 1999; Grabenbauer et al., 2003). Unfortunately, results regarding the prognostic and predictive significance of apoptosis are rather conflicting in SCCHN (Bartelink et al., 1999; Tsuchiya et al., 2001; Grabenbauer et al., 2003), which could be attributed, in part, to the methodologic complexities associated with these assays and the inherently asynchronous apoptotic process within a given...
Although EGFR overexpression seems to be predictive of response to lapatinib or lapatinib/CRT, EGFR gene amplification was not, which is in contrast to various reports with other EGFR inhibitors in SCCHN (Cohen et al, 2005; Erjrla et al, 2006; Agulnik et al, 2007; Thomas et al, 2007). Although no clear correlation was demonstrated between HER2 overexpression and response to lapatinib or lapatinib/CRT, a functional role through heterodimerisation with EGFR cannot be excluded. Of interest is that two of the four monotherapy responders showed both EGFR and HER2 coexpression. The patient with 3+ overexpression for both EGFR and HER2 also demonstrated gene amplification for both receptors. Similar to a previous study (Harrington et al, 2009), the treatment was well tolerated and did not lead to significant modifications of CRT. The majority of patients received the planned radiotherapy and chemotherapy, which was generally similar to that seen in other studies (Cooper et al, 2004). Furthermore, an independent data-monitoring committee, which evaluates the safety in several ongoing phase II and III trials of lapatinib in SCCHN, has raised no safety concerns in this regard.

The present study provides a useful design that allowed inter- and intrapatient evaluation of the pharmacodynamic effects and putative predictors of response to targeted agents. It showed the feasibility of obtaining paired biopsies in locally advanced SCCHN. However, some limitations of study design should be addressed. First, there is no consensus on the appropriate timing for the second biopsy; therefore, the timing for this study was empirically chosen. Hence, the observed effects may not reflect the actual molecular events that lead to apoptosis or growth arrest. Second, the small number of patients means the results should be interpreted with caution. Third, there is a fairly short follow-up period, which did not allow for assessment of survival.

In summary, a short treatment period with lapatinib suggests that it may 1) not affect apoptosis; 2) lead to inhibition of pEGFR; 3) decrease proliferation; 4) induce tumour regression and enhance response to CRT; and 5) cause oropharyngeal and oral cavity tumours to respond favourably to treatment. It would be of value to confirm these hypotheses with large cohorts of patients. Work is ongoing to investigate other EGFR family members and their effect on downstream signalling pathways.

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