



International Journal of Hepatology & Gastroenterology

Research Article

Efficacy and Safety of Erlotinib Addition to Concurrent Chemoradiation in Patients of Unresectable Esophageal Carcinoma: a Comparative Study -

Shyamji Rawat and Hemu Tandan*

Department of Radiotherapy, N.S.C.B. Medical College and Government Hospital, Jabalpur, 482003, India

***Address for Correspondence:** Hemu Tandan, Department of Radiotherapy, N.S.C.B. Medical College and Government Hospital, Jabalpur, 482003, India, Tel: +09827401465; ORCID: orcid.org/0000-0001-6219-6764; E-mail: hemusavtritandan@gmail.com, drshyamjirawat@yahoo.co.in

Submitted: 14 January 2018 **Approved:** 16 February 2018 **Published:** 19 February 2018

Cite this article: Rawat S, Tandan H. Efficacy and Safety of Erlotinib Addition to Concurrent Chemoradiation in Patients of Unresectable Esophageal Carcinoma: a Comparative Study. *Int J Hepatol Gastroenterol.* 2018; 4(1): 024-028.

Copyright: © 2018 Tandan H, et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



ABSTRACT

Background: Erlotinib is an oral EGFR Tyrosine Kinase (TK) inhibitor. Clinical trials of Erlotinib in combination with concurrent chemoradiotherapy in unresectable esophageal carcinoma have demonstrated improved clinical outcomes.

Purpose of study: We have prospectively evaluated the efficacy and safety of Erlotinib with Concurrent Chemoradiotherapy (CRT) in unresectable esophageal carcinoma compared with standard CRT.

Methods: In this prospective, two arm, comparative study, total 50 unresectable esophageal carcinoma patients received either Erlotinib (150 mg/day) with CRT or standard CRT. Treatment of CRT included cisplatin 50 mg/m² intravenously weekly concurrently with external beam radiation therapy. Tumor response was assessed as per RECIST v 1.1 criteria. Toxicity and Adverse Events (AEs) were assessed as per CTCAE v 4.

Results: The higher number of patients achieved complete response in the Erlotinib plus CRT group than the CRT group [14/25, 56% vs. 10/25, 40%, $p = 0.248$], but it was statistically not significant. The adverse events commonly encountered in both the treatment groups were majority of grade 1/2/3. A higher incidence of skin reaction hypocalcaemia and GI toxicity was noted in the Erlotinib plus CRT group in comparison to CRT. No grade IV and V toxicity were observed in Erlotinib with CRT. Erlotinib was observed to be safe with few manageable toxicity profiles.

Conclusion: The addition of Erlotinib to cisplatin based concurrent chemoradiotherapy resulted in mild improvement in the tumor response and was found to be feasible and safe in unresectable esophageal carcinoma.

Keywords: Erlotinib; Esophageal; Gastro-esophageal junction; Carcinoma; EGFR; Tyrosine kinase inhibitor

INTRODUCTION

Worldwide, esophageal cancer is the eighth most common cancer with an annual incidence of 456,000 new cases [1]. It is the fourth most common cause of cancer-related deaths in India [2]. The incidence of esophageal carcinoma varies widely by geographical location. In Asian-belt, the predominant histological type is squamous-cell carcinoma, whereas adenocarcinoma is predominant in western countries [3].

Currently, squamous-cell carcinoma is the most common type of esophageal cancer in the Indian subcontinent and the most common location is the distal third of the esophagus. The etiological factors for SCCs show a regional variation in different parts of India, but tobacco consumption in various forms, alcohol, hot beverages, and poor nutrition remain the predominant predisposing factors [2]. At presentation, the overwhelming majority of patients have locally or regionally advanced or disseminated esophageal cancer and have poor prognosis [2].

Chemoradiotherapy has now become the standard treatment in patients with unresectable, locally advanced esophageal cancer, but is associated with poor local-regional tumor control, risk of toxicities and the 5 year survival being less than 20% [4-6]. Many patients are not able to tolerate surgery and combined chemoradiation may be more appropriate in selected patients. But, local recurrence remains a common problem in this patients. Therefore, it is necessary to explore more effective treatment regimens so as to improve the therapeutic ratio and balance the associated toxicity.

Epidermal Growth Factor Receptor (EGFR) is overexpressed in approximately 30-70% of esophageal carcinomas and implicated in tumor genesis. Its overexpression has been correlated with poor patient prognosis and inferior response to conventional treatment [7,8]. Therefore, EGFR represents a valid target and anti-EGFR therapies are being explored to improve therapeutic outcomes in esophageal carcinoma.

Erlotinib is an oral and well tolerated drug that reversibly binds to the intra-cellular catalytic domain of EGFR tyrosine kinase, thereby

reversibly blocking EGFR phosphorylation, the signal transduction events and tumorigenic effects associated with EGFR activation [9,10].

In a phase II study of patients with gastro-esophageal junction/cardia and distal gastric adenocarcinoma, the activity of Erlotinib as monotherapy in esophageal cancer was modest [11]. Early phase I & II trial of Erlotinib in combination with concurrent chemoradiotherapy in esophageal carcinoma have demonstrated improved therapeutic outcome and survival benefit with the manageable mild toxicity profile [12,13]. These initial results suggest that this regimen has potential to enhance local control and improve survival in patients with esophageal cancer. Therefore, the present comparative study was carried out to evaluate the efficacy and safety of Erlotinib (150 mg/day) with concurrent chemo-radiotherapy in patients with unresectable esophageal carcinoma and compared with the concurrent chemo-radiotherapy alone.

MATERIALS AND METHODS

This was a prospective, two arm, comparative study carried out in patients with locally advanced esophageal carcinoma, attending the Jadao Ba Cancer Hospital, N.S.C.B. Medical College & Hospital Jabalpur (India) during the period of March 2015 to September 2016. The study was approved by the Institutional Ethical Committee and conducted in accordance with Good Clinical Practice guidelines and the Declaration of institutional committee.

The study included patients with the following eligibility criteria

- 1) Unresectable esophageal and gastro esophageal junction carcinoma either locally advanced or inoperable due to medical contraindication
- 2) Histopathologically proven squamous cell carcinoma or esophageal adenocarcinoma
- 3) T1-4 N1-3 M0 stage, according to TNM system
- 4) Age above 18 years, and 5) Eastern Cooperative Oncology Group performance (ECOG) status of 0, 1, or 2.



We excluded the following patients

- 1) Age \leq 18 years,
- 2) Previous treatment of esophageal carcinoma with surgery, radiotherapy, chemotherapy, or antineoplastic biological therapy,
- 3) Presence of severe co morbidities that will put the patient at a significantly higher risk or will damage the protocol compliance,
- 4) History of allergy with similar biological to Erlotinib/Cisplatin,
- 5) Evidence of metastases,
- 6) Presence of aero digestive fistula (trachea and/or bronchia),
- 7) Other synchronous malignancies,
- 8) Inadequate hematologic, cardiac, renal and hepatic functions,
- 9) Uncontrolled infection/any other systemic diseases,
- 10) Not willing to give informed consent,
- 11) Pregnant and lactating females.

Before enrollment, all patients gave a full history and underwent a physical examination. Routine blood investigation- Complete blood count with differential, electrolyte assessment, liver and renal function tests were done. Radiological investigation- Chest X-ray, electrocardiogram, USG abdomen, Upper Gastrointestinal Endoscopy (UGIE) and CT scan with contrast- neck/ thorax/ abdominal scan were done.

Two treatment groups (Test group and Control group) were defined. Patients were randomly allocated to either group to receive the treatment. Test group received Erlotinib plus concurrent Cisplatin-Based Chemoradiotherapy (CRT) treatment, while the Control group received only concurrent cisplatin-based chemoradiotherapy.

In the Control group, patients received cisplatin 50 mg/m² intravenously weekly concurrently with External Beam Radiation (EBRT). Patients in the study arm received daily Erlotinib 150mg plus cisplatin 50 mg/m² intravenously weekly concurrently with EBRT.

Radiotherapy treatment protocol schedule (both treatment groups)

Cases were treated by with an initial AP/PA approach up to 50Gy followed by three field technique [AP/ Right Posterior Oblique (RPO)/ Left Posterior Oblique (LPO)] up to 60Gy.

Radiotherapy- Total dose, 60Gy, 2Gy/#, 5 #/week was administered to all patients

CONCURRENT CHEMOTHERAPY PROTOCOL SCHEDULE

Control group

Cisplatin 50 mg/m² IV weekly: In the control group, patients received weekly Cisplatin 50 mg/m² IV in 300 ml Normal Saline over one hour. Premedication with antiemetic was given, with adequate hydration for two hours before and after the chemotherapy.

Test group

Daily Erlotinib 150mg OD plus Cisplatin 50 mg/m² IV weekly: In the Test group, patients received daily tablet Erlotinib 150 mg/ day

PO in empty stomach and was started from the first day of radiation and continued until the last day of irradiation. Along with this, weekly Cisplatin 50 mg/m² IV in 300 ml normal saline was started from day 1 of radiation. Ryle's tube insertion and esophageal stenting were the supportive measures that we provide to our grade III-IV dysphagia patients before the treatment.

Patients in both control & test group receiving CRT were assessed weekly for symptomatic, clinical improvement and adverse effects. During the study, patients were hospitalized when needed. Patients were assessed weekly and when required for toxicities arising from combined modalities. The primary response was assessed after one month of treatment completion.

Parameters evaluated: The Primary tumor response in both the groups was evaluated using the Response Evaluation Criteria in Solid Tumors (RECIST version 1.1) criteria. The tumor response outcomes assessed included Complete Response (CR), Partial Response (PR), Progression of Disease (PD), and Stable Disease (SD). Adverse Events in both the groups were assessed and graded by Common Toxicity Criteria for Adverse Events (version 4.0) criteria.

STATISTICAL ANALYSIS

Statistical analysis was performed with software (SPSS, version 20). Descriptive statistics were used to express the data findings. For categorical variables, Chi square or Fischer exact test was used as appropriate. P values \leq 0.05 was considered statistically significant.

RESULTS

A total of 50 patients of locally advanced esophageal carcinoma were enrolled in the present comparative study. The study comprised of two groups, Test group and Control group. In each group 25 patients were enrolled. The mean age of the patients in the Test group was 53.2 \pm 11.1 years and 52.1 \pm 10.2 years in the Control group. Majority patients in both the groups were males in comparison to females; the majority had squamous cell carcinoma and ECOG status of 1. The baseline characteristics of both group patients are summarized in Table 1.

Tumor response

We observed that higher number of patients achieved Complete Response (CR) in the Test group (Erlotinib with CRT) than in the Control (CRT) group [14/25, 56% vs. 10/25, 40%], whereas a higher number of patients achieved a Partial Response (PR) in the Control group compared to Test group. In our study we have noted that squamous cell variant responded well to treatment in both arms but adenocarcinoma variant responded poorly in both arms. The treatment responses observed between the two groups were not statistically significant (Table 2).

Safety and toxicity

All adverse events commonly encountered in Test group and Control group were of grade I /II /III. A higher incidence of skin reaction hypocalcaemia and GI toxicity was observed in the Test group (Erlotinib with CRT) in comparison to the Control Group (CRT), whereas neutropenia and vomiting were comparable in the both groups (Table 3). No grade IV and V toxicity were observed in the Test group (Erlotinib with CRT). Erlotinib administration was observed to be safe with few manageable toxicity profiles.

DISCUSSION

The present comparative study indicates that, the addition of Erlotinib to concurrent chemoradiotherapy resulted in mild improvement in the tumor response compared to concurrent chemoradiotherapy alone in patients with unresectable esophageal carcinoma.

Epidermal Growth Factor Receptor (EGFR) signaling is one of the key molecular pathways involved in the regulation of cell proliferation, cell differentiation, and tumor genesis. EGFR overexpression is found in approximately 30-70% of patients with esophageal carcinoma and associated with a poor prognosis [8-10]. Thus, EGFR signaling plays a pivotal role in the pathophysiology of esophageal carcinoma [8,10]. This affords a potential opportunity for anti-EGFR agents to improve treatment outcomes.

Erlotinib is an oral EGFR Tyrosine Kinase (TK) inhibitor that reversibly competes with ATP for binding the tyrosine kinase domain of EGFR, thereby reversibly blocking EGFR phosphorylation, the signal transduction events, and tumorigenic effects associated with

Table 1: Baseline characteristics of unresectable esophageal carcinoma patients in the treatment groups.

Characteristics		Erlotinib plus Concurrent chemo radiotherapy (Test group = 25)	Concurrent chemo radiotherapy (Control group = 25)
Age in years (%)	Mean (± SD)	53.2 ± 11.1	52.1 ± 10.2
Age Group in years (%)	18 -28	0 (0)	1 (4)
	29 -38	2 (8)	1 (4)
	39 - 48	7 (28)	7 (28)
	49 - 58	6 (24)	8 (32)
	59 - 70	10 (40)	8 (32)
Gender (%)	Male	15(60)	17 (68)
	Female	10 (40)	8 (32)
Performance status (%)	ECOG 0	5 (20)	4 (16)
	ECOG 1	16 (64)	17 (68)
	ECOG 2	4 (16)	4 (16)
Addictions (%)	Tobacco	17 (68)	18 (72)
	BIDI	7 (28)	11(44)
	Cigarette	2 (8)	1(4)
	Alcohol	6 (24)	9 (36)
Primary site of tumor in esophagus (%)	Upper 1/3 rd	11 (44)	7 (28)
	Middle 1/3 rd	7 (28)	8 (32)
	Lower 1/3 rd and GEJ	7 (28)	10 (40)
Tumour Stage (%)	II	11 (44)	9 (36)
	III	14 (56)	16 (64)
Histo-pathological (%)	Squamous cell carcinoma	23 (92)	21 (84)
	Adenocarcinoma	2 (8)	4 (16)

Table 2: Response to treatment in the Test group and Control group.

Response to treatment	Test group - Erlotinib plus Concurrent chemo radiotherapy Number (%)	Control group -Concurrent chemo radiotherapy Number (%)	P value
Complete Response (CR)	14 (56%)	10 (40%)	0.248
Partial Response (PR)	10 (40%)	14 (56%)	
Total*	24	24	

*Data unavailable for two patients (one in each group).

Table 3: Adverse events encountered in both the groups during the treatment.

Adverse events	Toxicity Grade	Test group - Erlotinib plus Concurrent chemoradiotherapy Number (%)	Control group - Concurrent chemoradiotherapy Number (%)
Neutropenia	1	5 (20 %)	03 (12%)
	2	5 (20%)	03 (12%)
	3	0	0
	4	0	0
	Total		10 (40%)
Hypocalcemia	1	9 (36%)	8 (32%)
	2	2 (8%)	1 (4%)
	3	3 (12%)	0
	4	0	0
	Total		14 (56%)
Vomiting	1	8 (32%)	4 (16%)
	2	9 (36%)	13 (52%)
	3	7 (28%)	5 (20%)
	4	0	0
	Total		24 (96%)
Diarrhoea	1	9 (36%)	5 (20%)
	2	7 (28%)	5 (20%)
	3	6 (24%)	0
	4	0	0
	Total		22 (88%)
Skin rash	1	9 (36%)	0
	2	2 (8%)	0
	3	0	0
	4	0	0
	Total		11 (44%)

EGFR activation [8-11]. Early phase clinical trials of Erlotinib with concurrent chemoradiotherapy in esophageal carcinoma have demonstrated favorable anti-tumor activity with manageable toxicity profile [12,13]. Therefore, the present study evaluated the safety and efficacy of concurrent chemoradiotherapy with or without Erlotinib in unresectable esophageal carcinoma.

In the present comparative study, we found that addition of Erlotinib to the concurrent chemoradiotherapy in unresectable esophageal carcinoma resulted in higher tumor response than concurrent chemoradiotherapy alone. However the difference in tumor response was not statistically significant. The higher number of patients achieved Complete Response (CR) in the Erlotinib with CRT group than in the CRT alone group [14/25, 56% vs. 10/25, 40%], the findings of improved tumor response with the addition of Erlotinib to CRT are similar to previous clinical trials [12-14]. However, in this study cisplatin-based chemotherapy was used instead of doublet chemotherapy.

In the phase 1 trial, Dobelbower et al. [12] evaluated the safety of Erlotinib in combination with radiation, 5-fluorouracil (5-FU) and cisplatin in patients with esophageal carcinoma. Patients received 50, 100 or 150 mg oral Erlotinib/day beginning on the first day of radiation (three patients in each dose cohort). Concurrent cisplatin (75 mg/m² i.v., days 8 and 36) and 5-FU (1000 mg/m² i.v., days 8-11 and 36-39) were also given with 50.4 Gy thoracic radiation, delivered at 180 cGy/day, 5 days/week. The major toxicities encountered were grade 1-2 diarrhea, grade 1 skin rash, grade 1-3 nausea and grade 3 dehydration. The phase I study demonstrated the safety and tolerability of Erlotinib delivered at 150 mg/day with concurrent 5-FU, cisplatin and thoracic radiation [12].

In the phase 2 trial, Li et al. [13] evaluated the feasibility and efficacy of addition of Erlotinib (150 mg daily) to concurrent paclitaxel and cisplatin and radiotherapy in 24 patients with locally advanced esophageal squamous cell carcinoma. Of the 24 patients, 11 (45.8%) attained a partial response, and 11 (45.8%) attained a complete response after treatment. The median follow-up of the 24 patients was 18.6 months (range, 7.1-29.6 months). The 2-year overall survival, local-regional control, and relapse-free survival were 70.1%, 87.5%, and 57.4%, respectively. During the chemoradiotherapy, the incidences of acute toxicities of Grade 3 or greater, such as leucopenia and thrombocytopenia, were 16.7% (4/24) and 8.3% (2/24). Skin rash was encountered in 21 patients on the face and chest, with 1 patient in Grade 3, 8 patients in Grade 2, and 12 patients with Grade 1. The study concluded that, addition of Erlotinib (150 mg daily) to concurrent chemoradiation yielded satisfactory 2-year overall survival and local-regional control in locally advanced esophageal squamous cell carcinoma [13].

Similarly, Zhao et al. [14] in a phase 2 trial investigated the efficacy and safety of addition of Erlotinib (150 mg daily) to concurrent (paclitaxel-based) chemoradiotherapy in patients with inoperable esophageal squamous cell carcinoma. Among the 21 patients treated, 8 (38.0%) achieved CR, 10 (47.6%) PR, and 3 (14.4%) SD. No patient showed Progressive Disease (PD). The median Local Progression-Free Survival (LPFS), Progression-Free Survival (PFS), and Overall Survival (OS) were 17.5 months, 14.3 months, and 22.9 months respectively. Two-year LPFS, PFS, and OS were 52.4%, 42.8%, and 67.0%, respectively. The most common side effects were esophagitis in 18 patients (85%), followed by hypoleukemia in 5 patients (23.8%), fatigue in 4 patients (19.0%), pulmonary toxicity in 3 patients (14.2%), and skin rash in 2 patients (9.5%). The study documented that the addition of Erlotinib (150 mg daily) to concurrent chemoradiation was effective, tolerated regimen for patients with inoperable esophageal squamous cell carcinoma [14].

In the present comparative study, the adverse events commonly encountered in both the treatment groups were majority of grade 1/2/3. The adverse effect profile observed was similar and comparable to findings observed previous studies [11-14].

A higher incidence of skin reaction hypocalcaemia and GI toxicity was observed in the Erlotinib with concurrent chemoradiation group in comparison to concurrent chemoradiation alone. Skin rash grade I was seen in 36% patients and grade II in 8% patients in Erlotinib treated group. It was adequately managed by administering Antihistaminics and emollients. Erlotinib is known to cause skin rashes as documented in previous studies, which were also seen in the present study. Hypocalcemia was managed by oral calcium and vitamin D3 supplements/ parenteral calcium supplementation. Diarrhea was managed by appropriate fluid replacement, anti-diarrheal agents, probiotics and ORS. In the present study, no grade IV and V toxicity were observed in Erlotinib with concurrent chemoradiation. Erlotinib was observed to be safe with few manageable toxicity profiles.

In summary, the addition of Erlotinib (150 mg/day) to concurrent chemoradiotherapy showed mild improvement in the tumor response in comparison to concurrent chemo-radiotherapy alone in squamous cell variant of unresectable esophageal carcinoma with manageable toxicity. Although robust multicenter, randomized control trials with larger sample size are warranted to validate these findings.

The study had limitations; the sample size was small, conducted

at a single hospital setting and short-term treatment outcomes were assessed. The long term safety, local recurrence, metastasis and overall survival benefit needs to be explored further.

CONCLUSION

The addition of Erlotinib (150 mg daily) to cisplatin-based concurrent chemoradiotherapy resulted in mild improvement in the tumor response and was found to be feasible and safe in unresectable esophageal carcinoma with few manageable toxicity profiles.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

REFERENCES

- Bray F, Jemal A, Grey N, Ferlay J, Forman D. Global cancer transitions according to the Human Development Index (2008-2030): A population-based study. *Lancet Oncol.* 2012; 13: 790-801. <https://goo.gl/tJLJWb>
- Samarasam I. Esophageal cancer in India: Current status and future perspectives. *Int J Adv Med Health Res.* 2017; 4: 5-10. <https://goo.gl/DEcmAes>
- Pennathur A, Gibson MK, Jobe BA, Luketich JD. Oesophageal carcinoma. *Lancet.* 2013; 38: 400-412. <https://goo.gl/pbW5yf>
- Zingg U, DiValentino D, McQuinn A, Mardzuki A, Thompson SK, Karapetis CS, et al. Outcome for esophageal cancer following treatment with chemotherapy and radiotherapy but not esophagectomy: Nonsurgical treatment of esophageal cancer. *Clin Exp Gastroenterol.* 2009; 2: 75-83. <https://goo.gl/gfGK3F>
- Zhu L-L, Yuan L, Wang H, Ye L, Yao GY, Liu C, et al. A meta-analysis of concurrent chemoradiotherapy for advanced esophageal cancer. *PLoS One.* 2015; 10: e0128616. <https://goo.gl/rr3jah>
- Cooper JS, Matthew D, Herskovic A, Macdonald JS, Martenson JA Jr, Al-Sarraf M, et al. Chemoradiotherapy of locally advanced esophageal cancer: Long-term follow-up of a prospective randomized trial (RTOG 85-01). *J Am Med Assoc.* 1999; 281:1623-1627. <https://goo.gl/LPqqy4>
- Kuwano H, Kato H, Miyazaki T, Fukuchi M, Masuda N, Nakajima M, et al. Genetic alterations in esophageal cancer. *Surg Today.* 2005; 35: 7-18. <https://goo.gl/ww1KLg>
- Pande AU, Iyer RV, Rani A, Maddipatla S, Yang GY, Nwogu CE, et al. Epidermal growth factor receptor-directed therapy in esophageal cancer. *Oncology.* 2007; 73: 281-289. <https://goo.gl/iJrusV>
- Moyer JD, Barbacci EG, Iwata KK, Arnold L, Boman B, Cunningham A, et al. Induction of apoptosis and cell cycle arrest by CP-358,774, an inhibitor of epidermal growth factor receptor tyrosine kinase. *Cancer Res.* 1997; 57: 4838-4848. <https://goo.gl/gWXVeb>
- Metha VK. Radiotherapy and Erlotinib Combined: Review of the Preclinical and Clinical Evidence. *Front Oncol.* 2012; 2: 31. <https://goo.gl/V9S93f>
- Dragovich T, McCoy S, Fenoglio-Preiser CM, Wang J, Benedetti JK, Baker AF, et al. Phase II trial of erlotinib in gastroesophageal junction and gastric adenocarcinomas: SWOG 0127. *J Clin Oncol.* 2006; 24: 4922-4927. <https://goo.gl/wFu9CE>
- Dobelbower MC, Russo SM, Raisch KP, Seay LL, Clemons LK, Suter S, et al. Epidermal growth factor receptor tyrosine kinase inhibitor, erlotinib, and concurrent 5-fluorouracil, cisplatin and radiotherapy for patients with esophageal cancer: A phase I study. *Anti-Cancer Drugs.* 2006; 17: 95-102. <https://goo.gl/45nwRJ>
- Li G, Hu W, Wang J, Deng X, Zhang P, Zhang X, et al. Phase II study of concurrent chemoradiation in combination with erlotinib for locally advanced esophageal carcinoma. *Int J Radiat Oncol Biol Phys.* 2010; 78: 1407-1412. <https://goo.gl/xm42a2>
- Zhao C, Lin L, Liu J, Liu R, Chen Y, Ge F, et al. A phase II study of concurrent chemoradiotherapy and erlotinib for inoperable esophageal squamous cell carcinoma. *Oncotarget.* 2016; 7: 57310-57316. <https://goo.gl/P7A4tX>